National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

University of Alberta

Multirate Estimation, Control and Monitoring of Fed-batch Fermentations

by

Ravindra Dheerendra Gudi ©

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in

Process Control

Department of Chemical Engineering
Edmonton, Alberta
Fall 1995

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

THE AUTHOR HAS GRANTED AN IRREVOCABLE NON-EXCLUSIVE LICENCE ALLOWING THE NATIONAL LIBRARY OF CANADA TO REPRODUCE, LOAN, DISTRIBUTE OR SELL COPIES OF HIS/HER THESIS BY ANY MEANS AND IN ANY FORM OR FORMAT, MAKING THIS THESIS AVAILABLE TO INTERESTED PERSONS.

L'AUTEUR A ACCORDE UNE LICENCE IRREVOCABLE ET NON EXCLUSIVE PERMETTANT A LA BIBLIOTHEQUE NATIONALE DU CANADA DE REPRODUIRE, PRETER, DISTRIBUER OU VENDRE DES COPIES DE SA THESE DE QUELQUE MANIERE ET SOUS QUELQUE FORME QUE CE SOIT POUR METTRE DES EXEMPLAIRES DE CETTE THESE A LA DISPOSITION DES PERSONNE INTERESSEES.

THE AUTHOR RETAINS OWNERSHIP OF THE COPYRIGHT IN HIS/HER THESIS. NEITHER THE THESIS NOR SUBSTANTIAL EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT HIS/HER PERMISSION.

L'AUTEUR CONSERVE LA PROPRIETE DU DROIT D'AUTEUR QUI PROTEGE SA THESE. NI LA THESE NI DES EXTRAITS SUBSTANTIELS DE CELLE-CI NE DOIVENT ETRE IMPRIMES OU AUTREMENT REPRODUITS SANS SON AUTORISATION.

ISBN 0-612-06216-3

Canada

University of Alberta

Library Release Form

Name of Author: Ravindra Dheerendra Gudi

Title of Thesis: Multirate Estimation, Control and Monitoring of Fed-batch Fermentations.

Degree: Doctor of Philosophy

Year this Degree Granted: 1995

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Ravindra Gudi
24, Abhinav Nagar,
Borivli(E), Bombay,
India - 400066

Dated August 4th, 1995

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Multirate Estimation, Control and Monitoring of Fed-batch Fermentations** by **Ravindra Dheerendra Gudi** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Process Control (Chemical Engineering).**

(Professor Sirish L. Shah)

(Professor Murray R. Gray)

(Professor Reginald K. Wood)

Z J Koles
(Professor Zoltan J. Koles)

WF Ramirez
(Professor W.F. Ramirez)
(External Examiner).

Date August 8, 1995

Dedicated, through my loving parents and gurujees, to the

Supreme Lord

Abstract

This thesis focuses on identification, control and monitoring of bioprocesses. In particular, it proposes strategies to incorporate infrequent and often delayed measurements of the primary process variables into traditional estimation and monitoring schemes. This incorporation results in algorithms that generate accurate estimates of the fermentation states. The work reported here also presents monitoring algorithms that give quick flagging of faults and good predictions of the final quality variable values.

Feedback regulation and control algorithms require (i) a good process model and (ii) regular sampling of the controlled process output. Bioprocesses usually involve several intermediate reactions and complex feedback mechanisms. It is therefore not possible to develop an accurate dynamic model for the bioprocess. A greater and more serious limitation from the control perspective is the lack of adequate on-line sensors that can measure the primary process variables on a regular basis. Thus, the task of regulation and monitoring is a formidable and challenging one.

Traditional estimation strategies address the problem of state and parameter estimation by using the (extended) Kalman filter in an inferential framework. Secondary measurements such as the carbon dioxide evolution rate (CER) are used along with an inferential model of the process to estimate the primary process outputs. Such strategies disregard the availability of the infrequent, delayed measurement of the primary process variables. To accommodate such measurements, this thesis proposes to use the multirate formulation of the classical Kalman filter. Observability problems due to measurement delays, that commonly occur due to elaborate off-line assay procedures, are addressed elegantly in the extended Kalman filter framework. Other observability problems due to inadequate modelling of the influence of nutrient concentrations on the specific growth rate are also shown to occur. These problems have been addressed by using a multirate formulation of a reduced order estimator that has been proposed in the literature. The adaptive estimation schemes proposed in this thesis have been extensively validated using simulations, experimental and industrial data. Nonlinear control, using the above adaptive estimator, along *a priori* specified trajectories is also demonstrated using simulations.

Often times, the only supervisory objective in process control is to perform the task of on-line monitoring and fault detection. This thesis proposes an approach based on the use of multivariate statistical tools, to perform monitoring and fault detection for fed-batch fermentation processes. The off-line measurements of the primary process variables are included using a multiblock, multirate formulation of the traditional projection to latent structures (PLS) algorithm. The resulting monitoring strategy is evaluated by extensive simulations.

Acknowledgements

This thesis is a cumulative effort of several people of which I consider myself to be the last. I would like to express my sincere thanks and gratitude to the following :

- My loving and affectionate parents who brought us up in the best way that is ever possible and made so many sacrifices to help us achieve, succeed and stand upon our feet; my brothers and their families for their constant support, love and encouragement.

- My supervisory committee (Professors Shah, Gray and Wood) who guided me through the coursework and research in the program; It has been a very pleasant and intellectually enriching experience working with them. To my supervisor, Professor Shah, I would like to express my sincere thanks and gratitude for his constant encouragement, advice and financial support. My research experience with Professor Shah has been extremely educative. Thank you Professor Shah for teaching me (i) to always keep the mathematics relevant to the application and (ii) to analyse complex problems by breaking them into simple ones. Professor Gray helped me in systematically analysing the control problems in fermentations from a biochemical viewpoint and was always willing to meet and participate inspite of his busy schedule as Department Chair and later as the Dean of FGSR. Thank you Professor Gray for the help with the experimental data and for your research inputs. The multirate aspect of this thesis was first conceived, along with my colleague Mr. N.Iyer, in a course offered by Professor Wood. I would like to express my gratitude to Professor Wood for this and also for painstakingly going through an earlier version of my thesis and pointing out ways to improve it.

- I would like to acknowledge and express my gratitude for the financial support and help from the Commonwealth Scholarship Program of the Department of Foreign Affairs and International Trade, Government of Canada. I would like to acknowledge the cooperation and help of the program officers of the Association of Universities and Colleges in Canada, the Canadian Bureau of International Education and the International

Council for Canadian Studies.

- Professor Denis Dochain (Universite Catholique de Louvain) and Professor Michel Perrier (University of Montreal) gave very useful comments and directions at an intermediate and critical stage of my thesis. I would like to express my gratitude to them. Professor Ray Rink of the Electrical Engineering Department (U. of Alberta) also deserves a special mention for the initial ideas on the multirate Kalman filter and for meeting, listening and offering suggestions at times when I was going nowhere. I would also like to thank Dr. P.K. Yegneswaran and the management of the Merck Pharmaceutical Company in the United States for the industrial data and useful comments on this work. Dr. Pradyumna Namdev (Schering Plough, New Jersey) also deserves a special mention for initiating me into the bioengineering area. I would also like to thank Professor K. Nandakumar for his timely advice and support during a critical time.

- Excellence in research is achievable only through extensive and intense interactions with fellow graduate students. I would like to make a special mention of my office buddy and colleague S. Lakshminarayanan for his help in this thesis research. I will definitely miss the musical environment in our office (and at home through Subhashree) and the active and intense research as well as the 'senti'-cup discussions. Pranob Banerjee, my ex-EIL buddy, also deserves a special mention for giving me a patient ear on all my (at most times stupid) ideas and for presenting my research paper in Australia. I would like to thank Professor Grant Fisher and all my colleagues in the Process Control Group at the University of Alberta -- present members (Dr. Lanre Badmus, Kent Qi, Mary Bourke, Munnawar Saudagar, Biao Huang, Chee Wong, Albert Chiu and Ricky Leung) and the past members (Dr. Ezra Kwok, Dr. Fengxi Zhou, Dr. Steve Niu, Randy Miller, Roman Walther, Viral Maniar, Shreekant Lalgudi, Sam Dhaliwal, Christine Ho, Natarajan Iyer) for maintaining the extremely high research standards and for the scholarly and intellectual environment in the Control Group meetings. It has helped to learn much more than what I did as a result of my thesis.

- I would like to express my sincere gratitude to Mr. Krishna and Latha Bhat, Drs

Vishwanatha and Vijaya Somayaji and family, all the members of the Kannada Cultural Association and our close friends in the Edmonton Community (Manisha and Srinivasan, Dr. Abhoy and Vineeta Ojha, Dr. Ramesh Sankaranarayanan, Mrs Lakshmi Ramanan, Mrs Sudha Gurjar and the Kembhavi family) for the affection and help showered on us and for making our stay here in Edmonton comfortable and pleasant.

■ Finally, I would like to express my gratitude to the very special and important person -- my loving and affectionate life partner Vasudha (Shruti) for her encouragement, patience and understanding. This thesis was really not possible without HER. I would also like to say a BIG thanks to my sweet little daughter Dhanvini for making her papa forget his research stress at home and for letting him work and complete his thesis.

TABLE OF CONTENTS

1. Introduction 1

   1.1 Motivation 1

   1.2 Thesis Outline 4

2. Optimal Estimation Strategies for Multirate Systems 6

   2.1 Introduction 7

   2.2 Plant Model 12

   2.3 Optimal Multirate Estimator Design 13

      2.3.1 Time Update Equation 14

      2.3.2 Measurement Update Equation 15

   2.4 Summary of the multirate Kalman filter algorithm 17

   2.5 Extensions 17

   2.6 References 18

   2.7 Nomenclature 19

3. Structural Observability Issues in State and Parameter Estimation. 22

   3.1 Introduction 23

   3.2 System Observability for Linear Time Invariant Systems 23

   3.3 System Observability for Nonlinear Systems 24

   3.4 Case Studies 26

      3.4.1 Case Study 1 : Fed-batch Fermentation reactor 26

      3.4.1 Case Study 2 : Nonlinear Chemical Reaction System 28

   3.5 Conclusions 36

   3.6 References 37

   3.7 Nomenclature 38

4. The Role of Adaptive Multirate Kalman Filter as a Software Sensor and its application to a bioreactor 41
4.1 Introduction 42
4.2 Model 44
4.3 Adaptive Multirate Kalman filter - a Unified Approach 46
4.3.1 Multirate Kalman filter 46
4.3.2 Model Estimation 48
4.4 Experimental Evaluation 51
4.4.1 Experimental System I : Fermentation of *Saccharomyces cerevisae* 51
4.4.2 Experimental System II : Fermentation of *Streptomyces clavuligerus* 59
4.5 Conclusions 65
4.6 References 65
4.7 Nomenclature 67

5. Development of a multirate EKF based state and parameter estimator 69
5.1 Introduction 70
5.2 Problem Formulation 73
5.3 Multirate Software Sensor 76
5.3.1 Multirate Iterated Extended Kalman Filter 76
5.3.2 Measurement delays 80
5.3.3 Comments on System Observability 82
5.4 Case Study 83
5.4.1 Estimator equations 84
5.4.2 Observability Analysis 85
5.4.3 Modified Observation System 89

5. 5.5 Estimation Results 92

5.6 Conclusions 98

5.7 References 101

5.8 Nomenclature 104

6. Multirate Adaptive Estimation in an Antibiotic Fermentation with delayed measurements 107

6.1 Introduction 108

6.2 Material and Methods 110

6.3 Process Modelling 112

6.4 Results and Discussions 119

6.5 Interpretation of Results 123

6.6 References 126

6.7 Nomenclature 129

7. Adaptive Multirate Estimation and Control of Nutrient levels in a Fed-batch Fermentation using off-line and on-line measurements 131

7.1 Introduction 132

7.2 Process Model 134

7.3 Estimator equations 137

7.4 Nonlinear feedback control 143

7.5 Estimation Results 144

- 7.5.1 Simulation Study 144
- 7.5.2 Verification using industrial data 153

7. 7.6 Control Performance 160
7.7 Conclusions 160
7.8 References 163
7.9 Nomenclature 167

8. Statistical monitoring of fed-batch fermentations 169
8.1 Introduction 170
8.2 System description and block diagram 173
8.3 The M3PLS algorithm 176
  8.3.1 Standard PLS regression 176
  8.3.2 Database structure for multirate batch monitoring 181
  8.3.3 M3PLS analysis 181
  8.3.4 Monitoring and online prediction with the M3PLS model 184
8.4 Results and Discussion 194
  8.4.1 Model building 196
  8.4.2 Monitoring a normal fermentation run 200
  8.4.3 Monitoring of abnormalities in the fermentation 207
8.5 Conclusions 218
8.6 References 219
8.7 Nomenclature 221

9. Conclusions and Recommendations 224
9.1 Conclusions 224
9.2 Recommendations for future work 226

Appendix A 228
  Dynamic model of a fed-batch fermentation 229
Appendix B 231
Maple source code and output for case study 1 232

List of Tables

Table #	Table Description	Page
5.1	Process and measurement noise covariance matrices for the simulation runs	95
6.1	Initial conditions and parameters used for the estimation runs	130
7.1	Measurement frequencies of the process variables for the industrial fermentation.	166
8.1	The NIPALS algorithm	179
8.2	The M3PLS algorithm	186
8.3	Relation between the M3PLS algorithm steps and the labels in Figure 8.5	189
8.4	Model Building step for the M3PLS algorithm	197

List of Figures

2.1 Multirate sampling in a pressurized flow box of a paper machine 8

2.2 Multirate sampling in a magnetic tape drive 8

3.1 Schematic diagram of the Nyquist-Ramirez reactor 30

4.1 Periodicity in the multirate Kalman filter (MKF) elements. 55

4.2 Profile of an estimated parameter for the fed-batch fermentation of *Saccharomyces cerevisae.* 56

4.3 State estimate profiles using the Multirate Kalman filter for the Saccharomyces system. 57

4.4 Exit gas profiles: MKF based filtering of the exit gas oxygen measurements for the Saccharomyces system. 58

4.5 The nutrient feeding profile for the antibiotic fermentation. 60

4.6 MKF state estimates for the antibiotic fermentation. 62

4.7 MKF based estimation of specific growth rate for the antibiotic fermentation. 63

4.8 MKF based estimation of the maintenance coefficient for the antibiotic fermentation. 64

5.1 Schematic diagram of multirate sampling for chemical processes. 75

5.2 Profiles of process variables and parameters from the simulation of a fed-batch antibiotic fermentation. 93

5.3 Performance of the multirate EKF based estimation strategy using simulated data. 94

5.4 Effect of an increase in the major sampling interval on the estimator performance. 97

5.5 Effect of a measurement delay of 30 sampling instants on parameter estimation. 99

5.6 Estimator performance on simulated data using a modified parameter covariance. 100

6.1 Experimental profiles for the cumulative biomass, the nutrient feeding policy and the CER for the fed-batch fermentation of *Streptomyces clavuligerus* NRRL 3885 120

6.2 Estimator performance in the presence of measurement delay using instantaneous CER measurements. 122

6.3 Estimator performance in the presence of measurement delay using modified measurement equation. 124

6.4 Estimator performance in the presence of measurement delay using modified sampling scheme in the transient region. 125

7.1 Block diagram for the biomass and substrate estimation algorithm. 142

7.2 Base case estimation: The state estimates generated by the estimator agree well with the values available from simulations. 146

7.3 Base case estimation: The parameter profiles generated by the estimator are quite close to the values specified in the simulations. 147

7.4 Effect of a poor initial guess of the yield coefficient on the state estimates. 149

7.5 Estimation of the yield coefficient starting from a poor initial guess. 150

7.6 Effect of measurement delays in the primary measurements on the state estimates. 151

7.7 Effect of measurement delays in the primary measurements on parameter estimation. 152

7.8 Effect of a changing yield coefficient on the substrate concentration estimates. 154

7.9 Adaptation of the yield coefficient. 155

7.10 Profiles of the state estimates for a changing yield coefficient, over the entire fermentation run. 156

7.11 Estimator validation using industrial data : State estimation. 158

7.12 Estimator validation using industrial data : Parameter estimation. 159

7.13 Nonlinear control of nutrient levels along specified trajectories. 161

7.14 Nonlinear control of nutrient levels in the presence of yield coefficient mismatch. 162

8.1 Block diagram of the measurement system for a fed-batch fermentation 174

8.2 Block diagram illustration of the iterations in the NIPALS algorithm 180

8.3 The database structuring for multirate monitoring in fed-batch processes 182

8.4 Logical structure and layout of the prediction and monitoring scheme 185

8.5 Datablock interactions and computations involved in the M3PLS algorithm 188

8.6 Model Building : Inner relationship plot for the first dimension 198

8.7 Model Building : Inner relationship plot for the third dimension 199

8.8 Model building step for the primary block 201

8.9 Model building step for the secondary block 202

8.10 Monitoring of a normal fermentation run via the IC block scores 203

8.11 Monitoring of a normal fermentation run via the primary block scores 204

8.12 Monitoring of a normal fermentation run via the secondary block scores 205

8.13 Quality prediction of a normal fermentation run 206

8.14 Monitoring of abnormality in an initial condition via the IC block scores 208

8.15 Monitoring of abnormality in an initial condition via the primary block scores 209

8.16 Monitoring of abnormality in an initial condition via the secondary block scores 210

8.17 Quality prediction for a batch having an abnormality in an initial condition. 211

8.18 Monitoring of a batch, with abnormalities in the sparging system, via the SPE plots 213

8.19 Monitoring of a batch, with abnormalities in the sparging system, via the secondary block scores 214

8.20 Quality prediction for batch with abnormalities in the sparging system 215

8.21 Detection of contamination by foreign microorganisms via the SPE plots 216

8.22 Detection of contamination by foreign microorganism via the secondary block scores 217

Chapter 1

Introduction

Bioprocesses involve the manufacture of low volume, high value specialty chemicals. The manufacturing process involves a proprietary recipe, is highly specialised and several parameters need to be optimized to achieve a productive batch run. There are significant incentives for implementing strategies that promote their optimal operation. Such optimization strategies however need careful monitoring and good control of process variables at optimal values. Bioprocesses have several deterrents in achieving optimized operation. These processes are poorly modelled, nonlinear, time varying and permit only infrequent sampling of the process due to the risk of contamination. There is also a significant measurement delay associated with off-line assay procedures. Bioprocesses therefore offer significant challenges in the development and implementation of optimization strategies that have proven to be so useful elsewhere in the chemical process industry. This thesis focuses on the issues related to estimation, control and statistical monitoring of biochemical processes. The results proposed in this thesis also have useful applications in traditional chemical processes such as polymerization and distillation.

1.1 Motivation

Feedback regulation of the process variables along *a priori* established optimal trajectories or profiles is one of the strategies that achieves optimal fermentation operation. Feedback control however requires regular sampling of the process variables; a requirement that is not easily possible in a bioprocess environment. It also needs a good model of the process that relates the controlled process outputs to the manipulated inputs. Due to the inability to model several reactions that take place in a fermentation, a lumped, qualitative model is used to describe the time evolution of the process states. This particular structural characterization results in a few process parameters such as the yield coefficient to be time varying. It also introduces a small plant-model mismatch. The nonlinear, time varying nature of such models and lack of knowledge regarding the exact dependence of critical process parameters such as the specific growth rate on the environmental variables, preclude the use of linear model based feedback regulation strategies. The above problems and other issues related to measurement, modelling, estimation and control in a bioprocess context are discussed in Fish *et al.*(1989).

Inferential estimation strategies, that use other secondary measurements along with a process model to generate estimates of the primary process variables, have been proposed for use in bioprocesses. Pioneering efforts in the area of state estimation for bioprocesses (Stephanopoulos and San, (1984), Bastin and Dochain (1990)) have shown that it is possible to use optimal inferential estimation strategies to generate estimates of the unmeasured critical process states such as the biomass and substrate concentrations from measurements of other secondary, growth related process outputs such as the carbon dioxide evolution rate (CER) and the oxygen uptake rate (OUR). In the work by Stephanopoulos and San (1984), an extended version of the optimal Kalman Filter (EKF) has been used along with a model based on material balances using an assumed chemical formula for the biomass. Estimation algorithms, such as the Kalman filter (Kalman (1960)), are termed optimal because they make use of all the information that is available about the process through the process model and the measurements and then determine the optimal estimate of the state variables in a minimum variance sense. Estimates are generated by optimally weighing the information available through the process model versus the measurements. In a typical bioprocess environment, in addition to the secondary variables, the primary variables are also sampled, although infrequently, for off-line monitoring. In such a scenario, estimation algorithms need to accommodate this infrequently sampled measurement to generate optimal estimates of the system states.

Thus, there is a need to incorporate the infrequently and irregularly sampled measurements of the primary process outputs into such estimation schemes. This thesis proposes a formal way of incorporating the infrequently measured, delayed primary system output into the estimation scheme by using a multirate extension of the optimal Kalman filter.

Observability issues are very important in the design of adaptive estimators. In fermentation processes (as shown by Stephanopoulos and San (1984)) as well as polymerization processes (as shown by Schuler and Suzhen (1985)), there indeed are a few state variables that are unobservable from the measurements. To generate estimates of such state variables, the strategy followed in both of the above processes, is to use reduced order estimators. Two subsystems are generated by partitioning the overall system into observable and unobservable susbsytems. The outputs (estimates) of the observable subsystem act as inputs to the unobservable subsystem which then generates open-loop estimates of the unobservable states by direct integration of the model equations. For the fed-batch fermentation, Stephanopoulos and San (1984) have shown that it is possible to generate estimates of the substrate concentrations, which are unobservable, from the measured CER. For the same problem, this thesis proposes to use the multirate Kalman filter to generate closed loop estimates of the substrate concentrations and the time varying yield coefficient.

Often times, batch or fed-batch fermentations are run by following a prescribed, time tested recipe. In such cases, feedback regulation and control is not really an important objective. It may not be necessary to explicitly generate estimates of the primary process variables. It is perhaps more important to monitor the fermentation using information that is available from the measured variable profiles of past, normal fermentation runs. Statistical process control strategies such as the principal component analysis (PCA) and projection to latent structures (PLS) can be used to analyze the resulting, often significantly correlated, data and perform early on-line detection of the occurrence of faults and deviations. Statistical monitoring strategies proposed so far (Nomikos and

MacGregor, (1994)) disregard the multirate nature of the measurement system so typical in bioprocesses. This thesis proposes a formal way of including such measurements into the monitoring scheme.

1.2 Thesis outline

This thesis is organized as follows. A detailed introduction and a summary of the chapter contributions is given at the beginning and end of each individual chapter. Each chapter also has a nomenclature section to describe the symbols used in the text of the chapter.

Chapter 2 looks at issues related to multirate sampling and presents the multirate extension of the classical Kalman filter that is necessary to accommodate multiple rates of sampling/ measurement availability into optimal estimation schemes.

The importance of a structural system observability analysis in the design of state and parameter estimation schemes is emphasized in chapter 3. Using case studies taken from the literature, it proposes a modified observation system that alleviates the problem of reduced system observability due to measurement delays typically found in off-line assay procedures in chemical and biochemical processes.

Chapter 4 focuses on the use of the multirate Kalman filter presented in chapter 2 with a sequential parameter estimation strategy illustrated by Park and Ramirez(1990) to perform adaptive estimation in fed-batch fermentations. Validation results using experimental data from two different fed batch fermentations is presented.

The use of an extended version of the multirate Kalman filter to perform simultaneous state and parameter estimation using infrequent, delayed off-line biomass measurements and regular, on-line secondary measurements, is presented in chapter 5. The strategy is evaluated via simulation results involving a fed batch antibiotic fermentation.

Chapter 6 looks at the effects of maintenance and endogenous metabolism on the adaptive estimation task. A modified system description and measurement equation has been used to alleviate the observability problems found to exist in such a scenario. The adaptive estimator is validated using experimental data from a fed batch fermentation.

A multirate, reduced order estimation scheme to account for unobservability of the substrate concentrations from on-line measurements, is presented in chapter 7. The multirate extended Kalman filter developed in chapter 5 is cascaded with a second multirate estimator to perform the estimation of substrate concentrations and the yield coefficient. Validation results using simulations and data from an industrial fermentation are presented.

Chapter 8 extends the multivariate statistical monitoring schemes, such as the Principal Component analysis and Projection to Latent Structures, proposed in literature to include off-line measurements and applies the resultant scheme to the monitoring of a fed batch antibiotic fermentation. The monitoring algorithm is evaluated using extensive simulations.

Chapter 9 presents conclusion from the results presented in the complete thesis and provides suggestions and directions for future research.

1.3 References

Bastin, G. and Dochain, D., "On-line estimation and adaptive control of bioreactors", Elsevier Science Publishing Co., Amsterdam, The Netherlands,(1990).

Fish, N.M., Fox, R.I. and Thornhill, N.F., " Computer Applications in Fermentation Technology", Elsevier Applied Science, New York, U.S.A., (1989).

Kalman, R.E., "A New approach to Linear Filtering and Prediction Problems", Trans. ASME Journal of Basic Engineering, **82**, p34-45, (1960).

Park, S. and Ramirez, W.F., "Optimal regulatory control of bioreactor nutrient concentration incorporating system identification", Chemical Engineering Science, **45**(12),p 3467, (1990).

Schuler, H. and Suzhen Z., "Chain length distribution in a polymerization reactor", Chemical Engineering Science, **40** (10), p1891, (1985).

Stephanopoulos, G. and San, K.Y.," Studies on on-line bioreactor identification", Biotechnology and Bioengineering, **26**, p1176,(1984).

Chapter 2.

Optimal Estimation Strategies for Multirate Systems

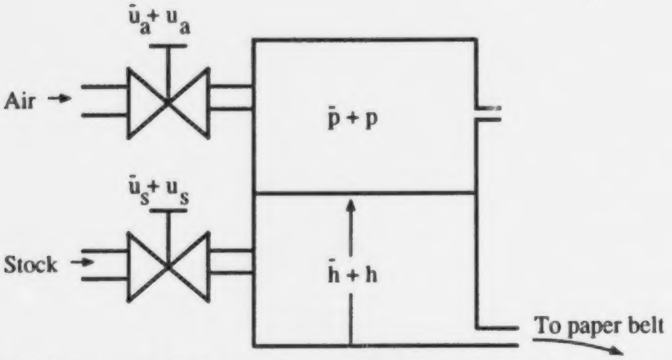
Filtering and estimation tasks for processes commonly encountered in chemical engineering need to formally accommodate multiple time scales and multiple rates of measurement availability. To achieve this objective, traditionally used optimal filtering and estimation algorithms need to be expressed in a multirate form. The main motivation of this chapter is the presentation of the linear Kalman filter in a multirate form. The algorithm equations and the filtering mechanism is discussed. Extensions needed to make the linear multirate Kalman filter applicable to processes in chemical and biochemical engineering, which are nonlinear and time varying, are also discussed.

2.1 Introduction

Traditional control schemes such as classical feedback control rely on regular sampling of the primary system output that needs to regulated. If the latter is not measurable at regular sampling times, other measurable secondary process outputs can be used along with an inferential model, that relates the primary and secondary process outputs, to generate estimates of the primary process output in some optimal sense. These estimates are then used to calculate control action updates. Even when measurements of the primary process outputs are available, they could be corrupted with measurement noise. This may necessitate the use of optimal filters to generate filtered estimates of the process output. Thus, traditional control and estimation schemes are designed to run at a uniform rate i.e. the system outputs are measured and the appropriate control action is computed at the same sampling rate.

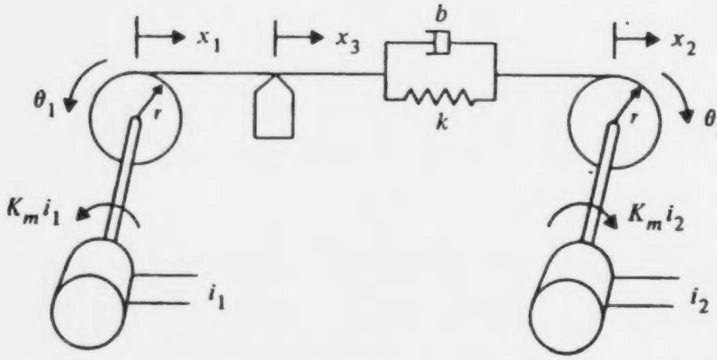
In multi-input, multi-output (MIMO) systems, it is sometimes helpful to use different sampling rates to obtain measurements of various system outputs and to calculate the control inputs. Such systems, with different rates of sampling for the measurements and control actions, are called multirate systems. Analysis of multirate systems was first done in the early 1950's (Crochiere and Rabiner,1983). During this time, the focus of the analysis was to convert the sampling rates of signals in the system from one rate to another with a view to increasing the efficiency of a processing algorithm. The need for multirate estimation and control was first felt in aerospace applications where due to high frequency bending mode effects, it was necessary to sample a few outputs at a rate that was an order of magnitude higher than that necessary for suitable control of rigid body motions (Glasson,1983). Multirate systems can occur broadly in two possible ways:

1. Uniform output rate, multiple input rate : In such systems, every output is measured at the same sampling rate but the input actions are calculated and implemented at a different sampling rate. One instance of this case is the paper machine example (Franklin *et al.*(1990)) shown in Figure 2.1. Because the air pressure dynamics are faster than the stock dynamics, the air rate control action needs to be implemented more rapidly than the



$\bar{u}_a + u_a$
Air
$\bar{p} + p$
$\bar{u}_s + u_s$
Stock
$\bar{h} + h$
To paper belt

Figure 2.1 : Multirate sampling in a pressurized flow box of a paper machine.



x_1
x_3
b
x_2
θ_1
r
k
r
θ_2
$K_m i_1$
$K_m i_2$
i_1
i_2

Figure 2.2 : Multirate sampling in a magnetic tape drive.

stock control action.

2. Uniform input rate, multiple output rate : In such systems, every input is sampled at the same rate but the outputs are measured at different sampling rates. For the magnetic tape drive (Franklin *et al.*(1990)) considered in Figure 2.2 , the tension in the tape *has* to be measured at a faster rate than the tape position, because the tape tension results from faster natural dynamics than the tape position.

The above examples illustrate naturally occurring MIMO systems where differing time scales/ dynamics require multiple rates of sampling. In many chemical/biochemical processes, a multirate sampling problem is also naturally posed, not only because of differing time scales, but often due to sensor limitations. The primary process outputs that need to be regulated, such as reactant/product concentrations in a reactor, cannot be measured on-line at a regular sampling time due to lack of adequate on-line sensors. They are usually sampled irregularly because of costly overheads in off-line assay procedures. The latter also introduces a significant measurement delay in the availability of the primary process variable values. Other secondary process outputs, such as reactor temperatures, that are strongly correlated with the primary process outputs, can be measured on-line at relatively rapid sampling rates. These secondary measurements are traditionally used, together with an inferential model, to generate estimates of the primary process variables. Thus a multirate sampling scenario exists in chemical/biochemical processes, even for systems with uniform time scales, due to lack of adequate on-line sensors for the primary process variables.

Conceptually, estimation schemes to estimate the states (and parameters) of a system from the measured outputs are said to be optimal when they make the best use of all the information that is available on the process model, the statistical properties of the process and measurement noise and the measured output data. Inferential estimation strategies that have been proposed so far in the literature for chemical processes do not accommodate the infrequently measured primary system output are therefore suboptimal with respect to the above optimality criterion.

Such state estimators can be considered optimal only if they are able to accommodate, in addition to regular measurements of the secondary process variables, the delayed and infrequent measurement of the primary process variables. In essence, such estimators must be able to accommodate multiple rates of sampling i.e the slow rates of sampling of the primary process variables and the relatively rapid rate of sampling of the secondary measurements. In addition, they must also be able to accommodate delays in the primary process measurements and compensate for the decreased system observability that is generally the consequence of delayed measurements.

Approaches to control infrequently measured process outputs via the inferential estimation route have been published extensively in the literature. Such strategies that have been proposed for linear systems cannot be directly applied to chemical processes because of the following characteristics :

(i) Process nonlinearities : For chemical processes, the interrelationships between the system states are usually nonlinear. Optimal state and parameter estimators thus need to be implemented in a linearized or a nonlinear framework to be applicable to chemical processes.

(ii) Time varying parameters: The kinetic parameters that appear in the chemical process model are not accurately known and are time varying. These time varying characteristics require the estimation scheme be implemented in an adaptive framework.

Estimation schemes, that do not accommodate the infrequently measured output, are usually based only on an inferential model and use only secondary measurements to estimate the primary process output (Brosilow and Tong, 1978). Such estimation schemes often give biased estimates of the process output because of model-plant mismatch and the presence of disturbances. Estimation schemes that incorporate the infrequently measured primary process output into estimation schemes were first proposed by D'Hulster and Cauwenberghe (1981). Guilandoust *et al.*(1987,1988) proposed a multirate estimation scheme that uses the infrequent primary measurement to update a transfer function based inferential process model which can then be used to estimate the primary variables from the secondary process outputs. Shen and Lee (1989) proposed an adaptive inferential control scheme for processes with intermittent measurements in which a discrete convolution model was directly adapted using the intermittent measurements. Lu and Fisher (1990) have formulated the multirate estimation problem such that the working equations reflect more fully and fundamentally the inferential relationships and have formally proved the convergence properties. Iyer (1992) derived the same transfer function model as a suboptimal case of the model that resulted from using the model based multirate Kalman filter formulation of Glasson (1980,1983). Tham *et al.* (1991) have implemented a multivariable, multirate self tuning control algorithm on a distillation column and have shown that improved performance can be obtained using the algorithm over a PI/PID based strategy. In all of the above approaches, the adaptation is carried out on a transfer function model of the process. While such an approach has inherent advantages with respect to global applicability and particularly for poorly modelled processes, it is useful to study the parameter adaptation to a dynamic state space model that results from a *structural* or qualitative description of the process. It is easy to relate the parameters of the model to the true process and any *a priori* process knowledge can be explicitly incorporated into the estimation scheme.

Kalman filtering strategies (Kalman, 1960) have been traditionally used to perform the tasks of filtering and inferential estimation of chemical processes. The process models used in the Kalman filter are state space models that result from writing a dynamic balance on the system states. The assumptions that need to be made in a Kalman filter formulation are (Maybeck (1979)):

(i) Linear model representation of the process : When nonlinearities exist, a typical engineering approach is to linearize about some nominal point to yield a linear perturbation or error model.

(2) Whiteness of the noise processes : This assumption can be justified from the point of view of mathematical tractability. Also, even if the noise is not white in the system bandpass, a shaping filter can always be used to achieve the representation of a linear

system driven by white noise.

(3) Gaussian distribution of the noise processes : This assumption can be justified by the fact that a process or measurement noise is typically caused by a number of small sources and it can be mathematically shown that when a number of independent variables are added together, the summed effect can be closely described by a Gaussian density.

For non-linear and (or) time varying processes, an extended Kalman filter is thus often used on a linearized model of the processes. When the system outputs are measured at different rates, the (extended) Kalman filter needs to be developed in a multirate framework.

Glasson (1980,1983) has proposed the multirate Kalman filter algorithm and has used it for estimation and regulation tasks in aerospace application. In this chapter, the multirate Kalman filter algorithm will be discussed and comments on extending this formulation to non-linear/time varying processes will be presented.

2.2 Plant Model

Consider the overall system state vector $\underline{X}$ (size n *by* 1) to be partitioned as $\underline{X}=[\underline{x}_p;\underline{x}_s]$ where $\underline{x}_p$ (size np *by* 1) and $\underline{x}_s$ (size ns *by* 1) are respectively the primary and secondary system states. The overall plant model can then be written in terms of the primary and secondary subsystems at any sampling instant i as,

$$\underline{X}(i+1) = \begin{bmatrix} \underline{x}_p(i+1) \\ \underline{x}_s(i+1) \end{bmatrix} = \begin{bmatrix} \underline{\underline{A}}_{pp} & \underline{\underline{A}}_{ps} \\ \underline{\underline{A}}_{sp} & \underline{\underline{A}}_{ss} \end{bmatrix} \begin{bmatrix} \underline{x}_p(i) \\ \underline{x}_s(i) \end{bmatrix} + \begin{bmatrix} \underline{w}_p(i) \\ \underline{w}_s(i) \end{bmatrix} \tag{2.1}$$

Let $\underline{v}$ (size nv *by* 1) and $\underline{y}$ (size ny *by* 1) be two independent measurement vectors associated with the measurement of the secondary and primary states respectively. The system output equations, in terms of the above measurement vectors, can be written as,

$$\underline{v}(i) = \left[\underline{\underline{H}}_v\right] X(i) + \underline{w}_v(i) \tag{2.2}$$

$$\underline{y}(i) = \left[\underline{\underline{H}}_y\right] X(i) + \underline{w}_y(i) \tag{2.3}$$

In the above equations, w is assumed to be a white, gaussian noise process. Let the measurement vector $\underline{y}$ correspond to the measurement of the primary states $\underline{x}_p$. These measurements would be available less frequently than the secondary measurement vector $\underline{v}$. This could occur because of sensor inadequacies in the measurement of $\underline{y}$ as explained earlier. Alternatively, it could also occur because of the relatively slower dynamics of a few of the states that affect $\underline{y}$. Both the measurement vectors need to be considered in developing an estimation strategy that optimally makes the best use of all information available about the process through process model and measured system outputs. Glasson (1980,1983) has presented an optimal multirate estimation algorithm that can formally accommodate the multiple rates of measurements related to the primary and secondary states. The estimation algorithm is discussed in detail in the following section :

2.3 Optimal multirate estimator design

Let us assume that the basic sampling period T to be the sampling period at which the secondary measurements arrive. We term those sampling instants when only the secondary measurements $\underline{v}$ are available as minor sampling instants. At other sampling instants, called the major sampling instants, we have measurements $\underline{y}$ and $\underline{v}$ associated with the primary and the secondary states. Let the major sampling instants arrive every JT time units, where J > 1 for a multirate scenario. The measurement vectors $\underline{z}_{major}$ ((ny+nv) *by* 1) and $\underline{z}_{minor}$ (nv *by* 1) at the major and minor sampling instants respectively can be written as,

$$\underline{z}_{major}(i) = \begin{bmatrix} \underline{\underline{H}}_y \\ \underline{\underline{H}}_v \end{bmatrix} \underline{X}(i) + \underline{w}_{major}(i) = \underline{\underline{H}}_{major} X(i) + \underline{w}_{major}(i) \tag{2.4}$$

$$\underline{z}_{minor}(i) = \left[\underline{\underline{H}}_v\right] \underline{X}(i) + \underline{w}_{minor}(i) = \underline{\underline{H}}_{minor} \underline{X}(i) + \underline{w}_{minor}(i) \tag{2.5}$$

It can be seen that the dimension of the measurement vector $\underline{z}$ varies periodically between the major and the minor sampling instants. Accordingly, the dimensions of the measurement noise covariance matrix varies periodically and can be denoted as $\underline{\mathrm{R}}_{z,major}$ ((ny+nv) *by* (ny+nv)) and $\underline{\mathrm{R}}_{z,minor}$ (nv *by* nv) at the major and minor sampling instants respectively. The dimensions and magnitudes of the Kalman filter gains derived by propagating the discrete Kalman filter covariance equation (Franklin *et al.*(1990)) to a (periodic) steady state are also periodic. The optimal multirate Kalman filter can then be written using the time update and measurement update mechanisms (Franklin *et al.*, 1990) as follows :

2.3.1 Time update equations

Assuming that an optimal estimate of the state vector $\hat{X}$(i-1/i-1) is available at the previous sampling instant, the time update equations can be written by simply integrating the model equations (2.1) as,

$$\underline{\hat{X}}(i/i-1) = \underline{\underline{A}}\ \underline{\hat{X}}(i-1/i-1) \tag{2.6}$$

$$\underline{\hat{y}}(i/i-1) = \underline{\underline{H}}_y\ \underline{\hat{X}}(i/i-1) \tag{2.7}$$

$$\underline{\hat{v}}(i/i-1) = \underline{\underline{H}}_v\ \underline{\hat{X}}(i/i-1) \tag{2.8}$$

The *a priori* (or before measurement) estimate of the covariance matrix of estimation errors $\underline{\mathrm{P}}$ is given by,

$$\underline{\underline{P}}(i/i-1) = \underline{\underline{A}}\ \underline{\underline{P}}(i-1/i-1)\ \underline{\underline{A}}^T + \underline{\underline{R}}_w \tag{2.9}$$

In the above equations, A is the state transition matrix defined in a partitioned form in equation (2.1) and $\underline{\mathrm{R}}_w$ is the covariance of the white, gaussian process noise shown in equation (2.1).

2.3.2 Measurement update equations

Since the dimensions of the measurement vector vary, independent update mechanisms need to be set up at the major and minor sampling instants. At the major sampling instants, the Kalman gain is a larger matrix (n *by* (ny+nv)) and can be written as :

$$\underline{\underline{K}}_{major} = \underline{\underline{P}}(i/i-1)\underline{\underline{H}}^{T}_{major}\left[\underline{\underline{H}}_{major}\underline{\underline{P}}(i/i-1)\underline{\underline{H}}^{T}_{major} + \underline{\underline{R}}_{z,major}\right]^{-1} \tag{2.10}$$

The measurement update equation can be written as,

$$\hat{\underline{X}}(i/i) = \hat{\underline{X}}(i/i-1)+\underline{\underline{K}}_{major}\underline{\varepsilon}_{major} \tag{2.11}$$

where ε_{major} is the innovation or estimation error at the major sampling instant and is given by :

$$\underline{\varepsilon}_{major}(i) = \begin{bmatrix} \underline{y}(i)-\hat{\underline{y}}(i/i-1) \\ \underline{v}(i)-\hat{\underline{v}}(i/i-1) \end{bmatrix} \tag{2.12}$$

The *a posteriori* (or after measurement) estimate of the covariance matrix of estimation errors $\underline{\underline{P}}$ is given by :

$$\underline{\underline{P}}(i/i) = \left[I - \underline{\underline{K}}_{major}\underline{\underline{H}}_{major}\underline{\underline{P}}(i/i-1)\right] \tag{2.13}$$

At the minor sampling instants the Kalman gain is a smaller matrix (n *by* nv) and is expressed as :

$$\underline{\underline{K}}_{minor} = \underline{\underline{P}}(i/i-1)\underline{\underline{H}}^{T}_{minor}\left[\underline{\underline{H}}_{minor}\underline{\underline{P}}(i/i-1)\underline{\underline{H}}^{T}_{minor} + \underline{\underline{R}}_{z,minor}\right]^{-1} \tag{2.14}$$

The measurement update equation at the minor sampling instant is given by :

$$\hat{\underline{X}}(i/i) = \hat{\underline{X}}(i/i-1) + \underline{\underline{K}}_{minor}\,\underline{\varepsilon}_{minor} \tag{2.15}$$

where ε_{minor} is the estimation error at the minor instants and can be written as,

$$\underline{\varepsilon}_{minor}(i) = \left[\underline{v}(i) - \hat{\underline{v}}(i/i-1)\right] \tag{2.16}$$

The propagated (*a posteriori*) value of the covariance matrix of estimation errors is :

$$\underline{\underline{P}}(i/i) = \left[I - \underline{\underline{K}}_{minor}\,\underline{\underline{H}}_{minor}\,\underline{\underline{P}}(i/i-1)\right] \tag{2.17}$$

The mechanism of the multirate estimation scheme can be explained in the context of a chemical reactor where the states of primary interest are the reactant/product concentrations which are generally measured at infrequent (major) sampling instants. The secondary measurement and state in this context is the temperature of the reactor contents which can be measured at regular (minor) sampling instants. The objective of the multirate estimation in the context of the chemical reactor is to generate estimates of the reactant/product concentrations and (filtered estimates of) the temperatures at regular sampling instants. At the minor sampling instants, when the primary measurements are not available, the estimates for the overall state vector are inferentially generated using the secondary measurements. This mechanism therefore requires the plant state vector to be fully observable from the secondary measurements. Even if the observability criterion is satisfied, the estimates can diverge for reasons such as mismatch in the inferential process model. Although this measurement update gives an optimal estimate of the state vector given the measurements at the minor sampling instant, the error covariance of the primary state grows with time. At the major sampling instant when the measurement of the reactant/product concentration is available, the primary state estimate is reset i.e. an accurate estimate of the primary state is possible with the primary measurement. These reset estimates are then propagated at subsequent sampling instants via the time update mechanism until a new primary measurement is available. The Kalman gains also exhibit a similar periodic behaviour. At the minor sampling instants, the Kalman gains associated with the innovations of the secondary measurements grow with time. The gains are reset when the primary measurements are available at the major sampling instants.

2.4 Summary of the multirate Kalman filter algorithm

Assuming that at any sampling instant i, a state prediction $\hat{X}(i/i-1)$ and a covariance matrix prediction $P(i/i-1)$, using data upto the previous (i-1) sampling instant, is available, the algorithm can be summarized as follows:

Step 1 Check if the sampling instant i is a major or a minor sampling instant. For automated sampling devices, i will be an integral multiple of JT (for example, i=5T,10T,....) at the major sampling instant and simply an integral multiple of T (for example, i=T,2T,3T,...) at the minor sampling instant. If the primary measurements arrive due to off-line assay procedures as in a chemical or a biochemical process, the arrival of such a measurement at the major sampling instant can be flagged by the operator.

Step 2 If i is a major sampling instant, perform the measurement update using equations (2.10-2.13). At the minor sampling instants, perform the measurement update using equations (2.14-2.17). The measurement update equations can be summarized as

- Generate the innovations vector by subtracting the measurement from the prediction (equation (2.12) or (2.16)).
- Evaluate the Kalman gain using the *a priori* covariance, the measurement noise covariance and the measurement matrices (equation (2.10) or (2.14))
- Generate the state estimate $\hat{X}(i/i)$ using equations (2.11) or (2.15).
- Evaluate the a posteriori estimate of the covariance of estimation errors (equation (2.13) or (2.17))

Step 3 Using equations (2.6) to (2.9) perform a one step ahead prediction of the state and the covariance matrix of estimation errors.

2.5 Extensions

The above multirate Kalman filter formulation has been developed for linear, time invariant systems. To make it applicable to a chemical process, it must be extended to be applicable to nonlinear processes and processes with time varying parameters. For nonlinear systems, it is often recommended that the system be linearized around some nominal operating point and use the same Kalman filter equations on the linearized system. The resulting filter is called the extended Kalman filter(EKF). Thus the linear formulation of the multirate Kalman filter can, in principle, be extended for multirate state estimation in nonlinear systems by formulating an extended multirate Kalman filter. For time varying parameters, several different approaches such as sequential parameter updating (Ljung and Soderstrom,1983) and the extended Kalman filter have been proposed. When time varying parameters are modelled as white noise processes with unspecified probability densities (Ray,1981), the resulting state space description is nonlinear. The task of adaptive estimation has been traditionally carried out using the EKF. For multirate systems, the time varying case can also be solved by using the extended multirate Kalman filter formulation. This will be discussed in greater detail in Chapters 3 and 5.

2.6 References

Brosilow, C.B. and Tong, M., "Inferential control of Processes: Part II. The structure and dynamics of Inferential control systems", AIChE Journal, **24**, p492-500, (1978).

Crochiere, R.E. and Rabiner, L.R., "Multirate Digital Signal Processing", Prentice-Hall Inc., Englewoods Cliff, New Jersey, USA (1983).

D'Hulster, F.M. and van Cauwenberghe, A.R., "Application of parameter adaptive inferential control to non-continuously measurable quality variables", Proceedings of the Second World Congress of Chemical Engineering, Montreal, Canada (1981).

Franklin, G.F., Powell, J.D. and Workman, M.L.," Digital Control of Dynamic Systems", Addison-Wesley Publishing Co., Reading, Massachussets, USA (1990).

Glasson, D.P., "Research in Multirate Estimation and control", Analytic Sciences Corporation, Six Jacob way, Reading , Massachussets, USA (1980).

Glasson, D.P., "Development and Applications of multirate Digital control", IEEE Control Systems Magazine,**3**(4),p2-8, (1983).

Guilandoust, M.T., Morris, A.J. and Tham, M.T., "Adaptive Inferential Control", IEE Proceedings, Pt. D, **134**(3), p171-179, (1987).

Guilandoust, M.T., Morris, A.J. and Tham, M.T., "An Adaptive Estimation algorithm for Inferential Control", Industrial and Engineering Chemistry, Process Design and Development, **27**, p1658-1664, (1988).

Iyer, N.M.,"Adaptive Inferential Control for Processes with Multirate Sampling", MSc thesis, University of Alberta, Edmonton, Canada (1992).

Kalman, R.E., "A New approach to Linear Filtering and Prediction Problems", Trans. ASME Journal of Basic Engineering,**82**, p34-45, (1960).

Ljung, L. and Soderstrom, T., "Theory and Practice of Recursive Identification", MIT Press, Cambridge, USA(1983).

Lu, W. and Fisher, D.G.," Multirate Constrained Adaptive Control", Int. Journal of Control, **51**, p1439-1456, (1990).

Maybeck, P.S., " Stochastic Models, Estimation and Control", Volume 1, Academic Press, New York, USA (1979).

Ray, W.H.,"Advanced Process Control", McGraw-Hill Book Co., New York, USA (1981).

Shen, G.C. and Lee, W.K., "Adaptive Inferential Control for Chemical Processes with Intermittent Measurements", Industrial and Engineering Chemistry Research, **28**,p557-563, (1989).

Tham, M.T., Vagi,F. Morris, A.J. and Wood R.K., " Multivariable and Multirate Self Tuning control : a Distillation column case study", IEE Proceedings, Pt. D, **138**(1), p9-24, (1991).

2.7 Nomenclature

Roman

i	sampling instant for discrete model representation.

Chapter 2: Optimal estimation strategies for multirate systems.

v	vector of secondary process measurements.
w	white noise vector.
x	state vector in the state space process description.
y	vector of primary process measurements.
z	measurement noise vector.
A	discrete state transition matrix.
H	measurement vector in the discrete system description.
K	Kalman gain matrix at any sampling instant.
P	covariance matrix of estimation errors.
PI	proportional-integral controller.
PID	proportional-integral-derivative controller.
R	covariance matrix of measurement noise.
X	superstate formed by appending the primary and secondary states.

Greek

epsilon	innovations vector at any sampling instant.

Superscripts

^	state estimate.

Subscripts

major	related to the major sampling instant.
minor	related to the minor sampling instant.
p	related to the primary subsystem.
pp	subscript used for appropriate partitioning of the state transition matrix in equation (2.1)
ps	subscript used for appropriate partitioning of the state transition matrix in equation (2.1)
s	related to secondary subsystem.
sp	subscript used for appropriate partitioning of the state transition matrix in equation (2.1)

ss	subscript used for appropriate partitioning of the state transition matrix in equation (2.1)
v	pertaining to the vector of secondary measurements.
w	pertaining to white noise process.
y	pertaining to the vector of primary measurements.
z	pertaining to the vector of measurements.

Chapter 3

Structural Observability Issues in Nonlinear State and Parameter Estimation

The main objective of this chapter is the investigation of methods to analyze and enhance the observability properties of nonlinear processes in a linearized framework. The concept of structural observability is introduced and a systematic procedure to perform the observability analysis is described and applied to two problems taken from chemical and biochemical engineering literature. Issues related to weak system observability due to the presence of measurement delays, that are commonly found in the measurement of chemical process variables, are also discussed and a simple yet effective way of modifying the measurement system to enhance the system observability in the presence of such measurement delays is presented.

3.1 Introduction

Optimal estimation schemes are often used when some of the system states of a process are not directly measurable but their values are required to provide regular control moves. The measured system output is usually a linear combination/function of the system internal states. For example, the measured product evolution rates, in a chemical reactor, are a function of the reactant concentrations. Alternatively, the measured system outputs can be one of the system states itself. In such cases, the need for optimal state estimation can also arise because the measurements could be corrupted with sensor noise. A noisy temperature measurement in a chemical reactor is one example of this case. From a process monitoring and control viewpoint, it is always desirable to have estimates of the system states that are generated by making best use of the system model and the measurements. In this sense, *optimal* estimates of the system states are required.

Central to all optimal estimation strategies is the assumption that the system states are observable from the measured system outputs. Conceptually, the system states must affect or influence the measured output in some known way in order for them to be observable from the measured outputs. Mathematically, the observability criterion can be verified by constructing an observability matrix and ensuring its full rank at all times. This chapter will briefly introduce the mathematical steps involved in constructing the observability matrix and verifying its rank for a linear time invariant system. These ideas will be extended to nonlinear systems. The concept of *structural system observability* will be introduced and applied to analyze the observability properties of

(i) Fed-batch fermentation process; and

(ii) A non-linear chemical reaction system with measurement delays.

3.2 System Observability for Linear Time Invariant Systems

Consider the following discrete state space model for a linear time invariant system,

$$\underline{x}(i+1) = \underline{\underline{\Phi}}\ \underline{x}(i) + \underline{\underline{\Gamma}}\ \underline{u}(i) + \underline{w}(i)\ ;\ E(\underline{w}\ \underline{w}^T)=Q \tag{3.1}$$

$$\underline{z}(i) = \underline{\underline{H}}\ \underline{x}(i) + \underline{v}(i)\ ;\ E(vv^T)=R_z \tag{3.2}$$

Let the system state vector $\underline{x}$ have n states and the measurement vector $\underline{z}$ have m measurements. The above system is said to be completely observable from the measurements for $t_0 \leq t \leq t_1$ if, for every t_0 and some t_1, every state $\underline{x}(t_0)$ can be determined from the knowledge of $\underline{z}(t)$ over the interval $t_0 \leq t \leq t_1$. Mathematically, the observability criterion can be verified by constructing the m *x* n *by* n observability matrix $\underline{\underline{O}}$ as,

$$\underline{\underline{O}} = \left[\underline{\underline{H}}\ ;\ \underline{\underline{H}}\ \underline{\underline{\Phi}}\ ;\ ......\ ;\ \underline{\underline{H}}\ \underline{\underline{\Phi}}^{n-1}\right] \tag{3.3}$$

For each of the states in $\underline{x}$ to be observable from the measurement $\underline{z}$, the rank of the above observability matrix must be n.

Remark 1 : The above observability test is a "yes" or a "no" measure. Ad-hoc measures of "strong" or "weak" observability can be obtained by examining the singular values or condition numbers of the observability matrix (Lin (1979), Shah *et al.*(1981)).

Remark 2 : The above observability criterion gives necessary conditions to achieve *exponential observability* for a given system. In other words, if the above criterion is satisfied, it is possible to achieve arbitrarily fast rate of convergence of the estimates to their true values by choice of the estimator gain. Even when the system is not exponentially observable, it is still possible to design observers whose performance (convergence characteristics) will depend on the experimental conditions. Such observers are called *asymptotic observers* (Bastin and Dochain,(1990)).

3.3 System Observability For Nonlinear Systems

For nonlinear systems, conditions to check for full system observability are difficult to apply relative to those for linear systems (Ray,1981). It is often recommended that the nonlinear model equation be linearized first about some nominal trajectory $\underline{\bar{x}}(t)$. The conditions that are used to verify full system observability for linear systems can then be applied on the linearized model (Hwang and Seinfeld, 1972). The results of such an observability analysis on linearized models will obviously be valid only in a local sense around the linearization point.

Consider the following continuous time, nonlinear process model,

$$\underline{\dot{x}}(t) = f(\underline{x},t) + \xi(t) \tag{3.4}$$

$$\underline{y}(t) = h(\underline{x},t) + \eta(t) \tag{3.5}$$

Linearizing around some trajectory $\underline{\bar{x}}(t)$ that satisfies (4), we get

$$\delta\underline{\dot{x}}(t) = A(t)\ \delta\underline{x}(t) \tag{3.6}$$

$$\delta\underline{y}(t) = H(t)\delta\underline{x}(t) \tag{3.7}$$

where,

$$\underline{\underline{A}}(t) = \frac{\partial f}{\partial \underline{x}}\ |\underline{\bar{x}}(t)\ ;\ \underline{\underline{H}}(t) = \frac{\partial h}{\partial \underline{x}}|\bar{x}(t) \tag{3.8}$$

In the above equations, the control input is ignored as it does not affect the observability analysis. Likewise, the noise processes are ignored because the objective of linearization is to check for observability and the latter is a property of deterministic systems (Ray, 1981). In order to check the observability in discrete time, we must discretize the system equations using a suitable sampling time τ. The resulting equations in the discrete domain at any sampling instant i can be written as,

$$\delta\underline{x}(i+1)=\Phi\ \delta\underline{x}(i) \tag{3.9}$$

$$\delta\underline{y}(i) = C(i)\delta\underline{x}(i) \tag{3.10}$$

In traditional estimation strategies, the linearization is done around the latest state estimate $\hat{\underline{x}}$(i). The observability matrix defined in equation 3.3 can be constructed using the above linearized system matrices and its rank can be checked to verify system observability. In principle, this exercise must be carried out on all possible values that the state vector $\underline{x}$ can take to ensure full system observability over the entire domain. This can be computationally very expensive. However, in practice, observability is found to depend quite strongly on the system structure (Ray, 1981). These two facts suggest the use of the concept of structural observability and symbolic computation tools to construct and check the rank of the observability matrix. The advantages to the symbolic approach are (i) structural deficiencies, either in the system description or in the measurement system that cause the system to be unobservable, can be detected easily without a knowledge of the actual process parameters and (ii) the results are *generic* i.e. they are valid over all values that the state vector $\underline{x}$ can take with the exception of zero and infinity. In this chapter, two case studies from the chemical engineering literature are considered to illustrate the symbolic approach. The symbolic computations were performed using Maple (Waterloo Maple Software,(1981)).

3.4 Case Studies

3.4.1 CASE STUDY 1 : Fed batch Fermentation

Consider the following dynamic equation for growth of biomass in a fed-batch fermentation process :

$$\frac{db(t)}{dt} = \mu(t)b(t) + w(t) \tag{3.11}$$

In the above equation, b is the cumulative biomass in mass units, μ is the specific growth rate of the microorganisms and *w* is a zero mean with unspecified probability distribution. Due to inadequacies of structural models for the specific growth rate as proposed in the literature (Bastin and Dochain,(1990)), its dynamics are assumed to be modelled by a white noise process as,

$$\frac{d\mu}{dt} = w_2(t) \tag{3.12}$$

where w_2 is again a zero mean white noise input with unspecified distribution. The specific growth rate is then estimated on-line. Let the measured system outputs be (i) cumulative biomass b and (ii) the carbon dioxide evolution rate(CER) which is a function of the growth and maintenance related culture activities. The system output equation can then be written as,

$$\underline{y}(i) = \begin{bmatrix} b(i) \\ CER(i) \end{bmatrix} + \eta(i) = \begin{bmatrix} b(i) \\ (k_1\mu(i)+m_c(i))b(i) \end{bmatrix} + \eta(i) \tag{3.13}$$

In the above equations, m_c is the maintenance coefficient and k_1 is the yield coefficient relating biomass growth to CER. The maintenance coefficient can be time varying for the class of antibiotic fermentations. Although it is not an important parameter in the fermentation, its value still needs to be updated in an estimator model to prevent bias in the specific growth rate estimates. Its dynamics are also assumed to be modelled by a white noise process as,

$$\frac{dm_c}{dt} = w_3(t) \tag{3.14}$$

3.4.1.1 Observability analysis

Our objective is to analyze the observability properties of an estimator model that can generate estimates of the cumulative biomass, the specific growth rate and the maintenance coefficient from the measurements of the cumulative biomass and CER. To do this, we construct an augmented state vector $\underline{X}=[b\ \mu\ m_c]$. The dynamics represented by the augmented state vector $\underline{X}$ (equations 3.11,3.12 and 3.14) is clearly nonlinear (bilinear) as it involves a product of two states of $\underline{X}$. Linearizing the above equations around the latest state estimate $\underline{\bar{X}}(i)$ and performing a simple Euler discretization with a sampling time τ, the discrete state transition matrix can be symbolically written as :

$$\underline{\underline{\Phi}} = \begin{bmatrix} 1+\tau\mu(i) & \tau b(i) & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{3.15}$$

The linearized measurement matrix $\underline{H}$ can be expressed as,

$$\underline{\underline{H}} = \begin{bmatrix} 1 & 0 & 0 \\ k_1\mu(i)+m_c(i) & k_1 b(i) & b(i) \end{bmatrix} \tag{3.16}$$

The structural observability matrix O=[H;HΦ;HΦ^2] can be symbolically computed as,

$$\underline{\underline{O}} = \begin{bmatrix} 1 & 0 & 0 \\ k_1\mu(i)+m_c(i) & k_1 b(i) & b(i) \\ (1+\tau\mu(i)) & \tau b(i) & 0 \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i)) & (k_1\mu(i)+m_c(i))\tau b(i)+k_1 b(i) & b(i) \\ (1+\tau\mu(i))^2 & \tau b(i)(2+\tau\mu(i)) & 0 \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i))^2 & (k_1\mu(i)+m_c(i)(2+\tau\mu(i)\tau b(i)+k_1 b(i) & b(i) \end{bmatrix} \tag{3.17}$$

Using Maple (source code and results shown in Appendix B), it can be shown that the above observability matrix has no structural deficiencies and is of full rank i.e. it has a rank of 3. Therefore, it can be concluded that the augmented system is observable from the measurements defined in equation 3.13.

3.4.2 CASE STUDY 2. A non-linear chemical reaction system

The Nyquist-Ramirez reactor has been extensively studied, in the literature, for estimation and control (Nyquist and Ramirez, 1971 , Lynch and Ramirez, 1975). The reaction that takes place in the reactor is the decomposition of hydrogen peroxide to oxygen and water:

H_2O_2 -----> H_20 (*l*) + 1/2 O_2 (*g*)

The reaction is catalysed by potassium iodide which is fed to the reactor with hydrogen peroxide. The reactor is shown in Figure 3.1. The mathematical model for this process is as follows (Ramirez, (1994)).

Peroxide Material Balance: The peroxide concentration (C) can be modelled as,

$$\frac{dC}{dt} = \frac{F_p C_0}{V_x} - \frac{FC}{V_x} - R \tag{3.18}$$

Catalyst Material Balance: The dynamics of the catalyst concentration (C_{KI}) is written as,

$$\frac{dC_{KI}}{dt} = \frac{F_{KI}}{V_x} C_{KI0} - \frac{C_{KI}F}{V_x} \tag{3.19}$$

Energy Balance: The temperature (T) of the reactor contents can be modelled as,

$$\frac{dT}{dt} = \frac{F(T_0 - T)}{V_x} + \frac{Q_s}{V_x \rho C_p} + \frac{(\Delta H) R V_x - \beta(T - T_c)}{V_H \rho C_p} \tag{3.20}$$

with the reaction rate (R) given as,

$$R = k_0 C_{KI} C e^{\frac{-E}{R'(T+273)}} \tag{3.21}$$

In its original formulation, the system has two measured outputs. One is the reactor temperature T which is also a state variable. Measurements of reaction rate R, which is a nonlinear function of the reactant states and temperature, are also available. Since the reactant concentrations are not directly measured online, an inferential estimation scheme is necessary to generate estimates of the reactant concentrations from the rate and temperature measurements. It has been shown (Lynch and Ramirez, 1975) that the above system is fully observable and the system states can be estimated inferentially using the rate and temperature measurements. The observability criterion is however only a necessary condition for the estimation scheme to work. Often, inferential estimation strategies can generate biased estimates of the states due to modelling errors. For routine monitoring, the primary states i.e. the reactant concentrations are measured irregularly,

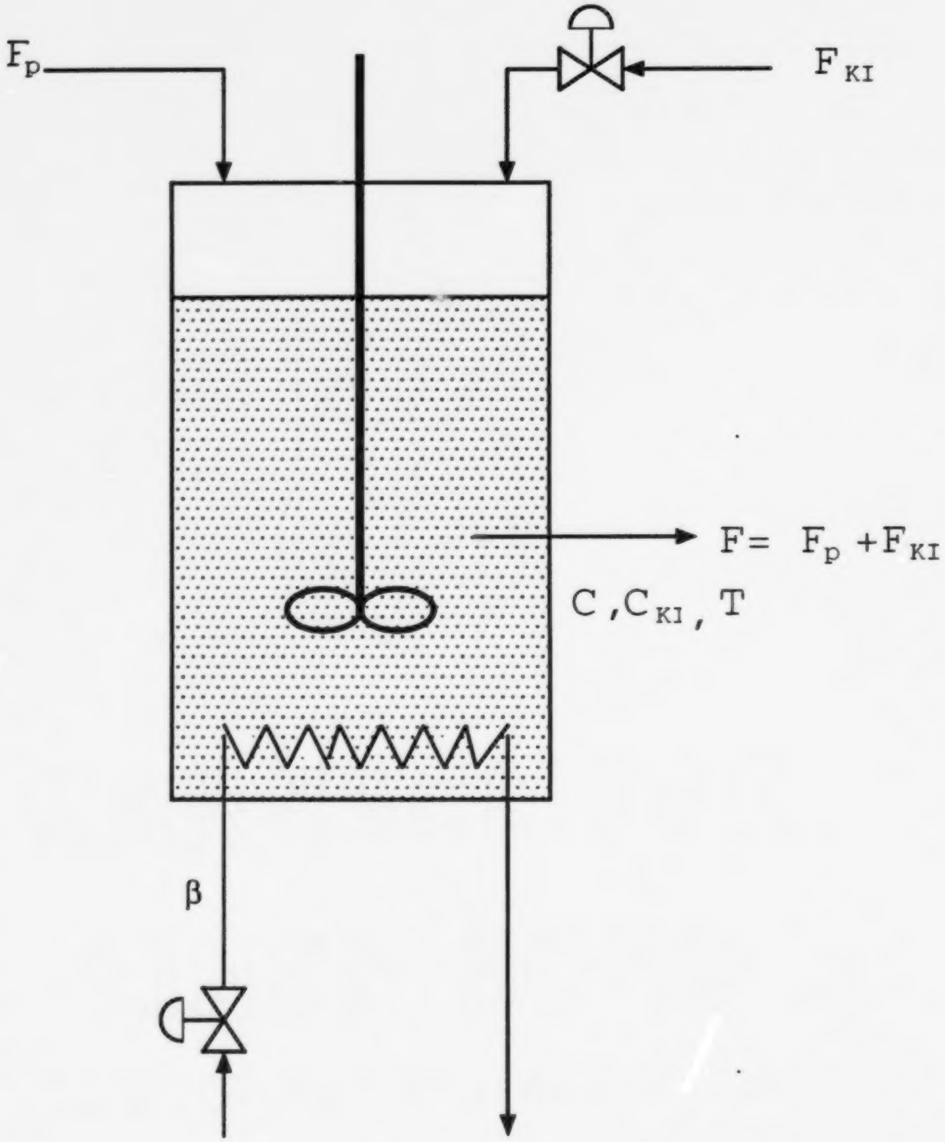

F_p
F_{KI}
$F = F_p + F_{KI}$
C, C_{KI}, T
β

Figure 3.1: Schematic diagram of the Nyquist-Ramirez reactor.

off-line and with some delay. These measurements can also be incorporated into the estimation strategy. As will be seen in later chapters, incorporation of the infrequent and delayed measurement of the primary process variables such as the reactant concentrations results in (i) generating closed-loop estimates of the state variables, and (ii) enhancement of the observability properties of the system in terms of improved condition number of the observability matrix. In this case study, we will analyze the observability properties of an estimator model in which, in addition to the temperature and rate measurements, delayed measurements of the reactant concentrations are also used in the estimation task. For simplicity, we assume that the catalyst concentration is maintained at a constant value by manipulating the catalyst feed rate using a perfectly tuned controller. The measurement delays in the reactant (peroxide) concentrations can be modelled by the addition of a fictitious state C_m as,

$$C_m(t) = C(t-t_m) \tag{3.22}$$

At instants when the peroxide concentration, temperature and rate measurements are available , the output equation can be written as :

$$y_{major}(t) = \begin{bmatrix} C_m(t) \\ T(t) \\ R(t) \end{bmatrix} + \nu_{major}(t) \tag{3.23}$$

We term these instants as the major sampling instants. At the remaining sampling instants, termed as the minor sampling instants, only the temperature and rate measurements are available and the measurement equation is expressed as,

$$y_{minor}(t) = \begin{bmatrix} T(t) \\ R(t) \end{bmatrix} + \nu_{minor}(t) \tag{3.24}$$

3.4.2.1 Observability analysis: In this section, we will perform an symbolic observability analysis for the system based on measurements at the major and minor sampling instants. The detailed symbolic matrices that were computed by Maple are not shown in the analysis here due to lack of space. Instead, a systematic procedure to perform the observability analysis is presented.

Step I: Linearization of state equations

We first introduce normalized dimensionless state variables as,

$$x_1 = \frac{(C-C_s)}{C_s} \; ; \; x_2 = \frac{(T-T_s)}{T_s} \tag{3.25}$$

where C_s and T_s are the steady state values of the peroxide concentration and temperature. Rewriting equations (3.18) and (3.20) in terms of the new state variables x_1 and x_2,

$$\frac{d}{dt}\begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \frac{dX}{dt} = \begin{bmatrix} f_1(x_1,x_2) \\ f_2(x_1,x_2) \end{bmatrix} = \underline{F}(X,t) \tag{3.26}$$

where f_1 and f_2 are the transformed dynamic balances of equations 3.18 and 3.20. Linearizing the above equations around the steady state, the linearized system of equations can be written in terms of the deviation variables as,

$$\delta\dot{X}(t) = A\delta X(t)$$

Discretizing the above equation with a sampling time τ and symbolically evaluating the matrix exponential during the discretization, the discrete state space equation can be written at any sampling instant i as,

$$\delta X(i+1) = \underline{\underline{\Phi}}\delta X(i) \tag{3.28}$$

Step II: Inclusion of delay states into the state equation

We now need to express the delayed states defined in equation 3.22. in the discrete domain. Assuming that the delay time t_m is an integral multiple of the basic sampling time

τ, we can write the delay equation 3.22 for the case of $t_m = 2\tau$ in terms of the deviation variables as,

$$\delta x_3(i+1) = \delta x_1(i) \qquad (3.29)$$

$$\delta x_4(i+1) = \delta x_3(i) \qquad (3.30)$$

In the above equations, x_3 and x_4 are the fictitious states that simulate the delay in the discrete domain. They can be appended to the system state vector X to form an augmented state vector X_d whose dynamics can be represented by,

$$\delta X_d(i+1) = \underline{\underline{\Phi}}_d \; \delta X_d(i) \qquad (3.31)$$

where Φ_d is given by,

$$\underline{\underline{\Phi}}_d = \begin{bmatrix} \phi_{11} & \phi_{12} & 0 & 0 \\ \phi_{21} & \phi_{22} & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \qquad (3.32)$$

Step III: Linearization of the measurement equation.

At the major sampling instants, the delayed measurement of the peroxide concentration is available. From equations 3.29 and 3.30, it is easy to see that x_4 represents x_1 delayed by 2 sampling instants. Thus x_4 represents the measurement C_m normalized according to (3.25). Also, the temperature and reaction rate measurements in (3.24), (3.25) can be expressed in terms of the normalized states x_1 and x_2. The measurement equation 3.23 and 3.24 can thus be re-written as,

$$y_{minor}(t) = G_{minor}(t) + \nu_{minor}(t) \; ; \; G_{minor} = \begin{bmatrix} x_2(t) \\ R(x_1(t), x_2(t)) \end{bmatrix} \qquad (3.34)$$

$$y_{major}(t) = G_{major} + \nu_{major} \;;\; G_{major} = \begin{bmatrix} x_4(t) \\ x_2(t) \\ R(x_1(t),x_2(t)) \end{bmatrix} \tag{3.33}$$

The above equations can be linearized and written in terms of the deviation variables of the augmented state X_d in the discrete domain as,

$$\delta y_{major}(i) = H_{major}\delta X_d(i) + \eta_{major}(i) \;;\; H_{major} = \frac{\partial G_{major}}{\partial X_d} \tag{3.35}$$

$$\delta y_{minor}(i) = H_{minor}\delta X_d(i) + \eta_{minor}(i) \;;\; H_{minor} = \frac{\partial G_{minor}}{\partial X_d} \tag{3.36}$$

Step IV : Symbolic construction of the observability matrix

The observability matrix can be symbolically constructed using the Φ_d and the H matrices at the major and minor sampling instants. The rank of the observability matrix can also be symbolically checked to verify full system observability.

3.4.2.2 Results of observability analysis:

The symbolic observability analysis outlined above was performed for the Nyquist-Ramirez reactor system. The symbolic computations were performed using the Maple software (Waterloo Maple Software, (1981)). The analysis indicated that at the major sampling instant, the structural observability matrix had a full rank (i.e. rank = 4) and so the system was fully observable from the measurements. Therefore at the major sampling instants, an estimation scheme to generate estimates of the state vector X_d using frequent rate and temperature measurements and infrequent and delayed composition measurements, can be developed. However at the minor sampling instant, the observability matrix was found to be rank deficient with a rank defect of 2. Thus only 2 of the states in the augmented state vector X_d were observable from the rate and temperature measurements defined in equation (3.34). Thus, the observation scheme defined by (H_{minor},Φ_d) was concluded to be *structurally deficient*.

3.4.2.3 Alternative measurement scheme The results of the observability analysis at the minor sampling instants indicate that the rank of the observability matrix for the system without measurement delay (equation 3.26) is equal to that for the delayed system description (equation 3.31). This indicates that the delayed states x_3 and x_4 that have been added to simulate the delay are unobservable from the measurements available at the minor sampling instants. Intuitively, the delayed states must influence the past system outputs and therefore must have some correlation with them. The latter fact also suggests that if the measurement equation is modified to include past inferential measurements of the reaction rates and temperatures, it may be possible to achieve full system observability at the minor sampling instants as well. Since the reaction rate is a function of reactor temperature which is also one of the states in the system description, we need to add delayed temperature states in the system description to be able to include past rate measurements in the measurement equation. The equations to include delays in temperature measurements can be expressed as,

$$\delta x_5(i+1) = \delta x_2(i) \tag{3.37}$$

$$\delta x_6(i+1) = \delta x_5(i) \tag{3.38}$$

In the above equations, x_5 and x_6 are states that are added to simulate a delay of 2 sampling instants for the temperature measurements. The dynamics of the augmented state vector $X_{d,m}$=[x_1 x_2 x_3 x_4 x_5 x_6] then become:

$$\delta X_{d,m}(i+1) = \underline{\underline{\Phi}}_{d,m}\, \delta X_{d,m}(i) \tag{3.39}$$

where $\Phi_{d,m}$ is given by,

$$\underline{\underline{\Phi}}_{d,m} = \begin{bmatrix} \phi_{11} & \phi_{12} & 0 & 0 & 0 & 0 \\ \phi_{21} & \phi_{22} & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix} \tag{3.40}$$

It was decided to analyze the system observability with the following modified measurement equation system at the major and minor sampling instants,

$$y_{major,\text{mod}} = G_{major,\text{mod}}(t) + \nu_{major,\text{mod}}(t) \; ; \; G_{major,\text{mod}} = \begin{bmatrix} x_4(t) \\ x_2(t) \\ x_5(t) \\ x_6(t) \\ R(x_1(t),x_2(t)) \\ R(x_3(t),x_5(t)) \\ R(x_4(t),x_6(t)) \end{bmatrix} \tag{3.41}$$

$$y_{minor,\text{mod}}(t) = G_{minor,\text{mod}}(t) + \nu_{minor,\text{mod}}(t) \; ; \; G_{minor,\text{mod}}(t) = \begin{bmatrix} x_2(t) \\ x_5(t) \\ x_6(t) \\ R(x_1(t),x_2(t)) \\ R(x_3(t),x_5(t)) \\ R(x_4(t),x_6(t)) \end{bmatrix} \tag{3.42}$$

3.4.2.4 Results of the observability analysis

The structural observability analysis on the above modified system was performed symbolically using Maple. It showed that the observability matrix was of full rank (i.e. rank = 6) and had no structural deficiencies at both, the major as well as at the minor sampling instant. Thus, use of the modified measurement equations 3.41 and 3.42 enabled formal incorporation of the infrequently available, often delayed measurement of the primary process state into the estimation scheme.

3.5 Conclusions

Conditions for full system observability are difficult to verify for nonlinear systems. Symbolic computation tools such as Maple prove to be very useful in analyzing the observability properties of nonlinear systems because (i) it is easy to perform the tasks of linearization and rank determination symbolically rather than numerically and (ii) it is relatively trivial to detect structural deficiency in the system. This chapter considered two case studies that are commonly encountered in chemical engineering. Numerical methods to ensure full rank of the observability matrix over the entire range of values that the states and parameters can take are computationally intensive and need *a priori* information regarding the expected values of the states and parameters. The structural observability study presented in this chapter that is based on the use of symbolic computation tools is more practical and useful as it does not need any such *a priori* information and is relatively easy to perform. Its results are valid in a *generic* sense over the entire range of values that the states and parameters can take (with the exception of zero and infinity). The analysis of the nonlinear chemical reaction system has shown that it is possible to enhance system observability by suitably modifying the measurement system. For chemical reaction systems, which usually have infrequent and significant measurement delays in the primary variables, the alternative measurement system proposed in equations (3.41) and (3.42) is very useful to formally incorporate the irregular, delayed primary measurement into an optimal estimation scheme.

3.6 References

Bastin, G. and Dochain, D., "On-line estimation and adaptive control of bioreactors", Elsevier Science Publishing Co., Amsterdam, The Netherlands,(1990).

Hwang, M. and Seinfeld, J.H., "Observability of nonlinear systems", Journal of Optimization Theory and Applications, **10**(2), p67-77, (1972).

Lin, C.T., "Structural Controllability", IEEE Transactions on Automatic Control, **19**, p201, (1974).

Lynch, E.B and Ramirez, W.F., "Real-time Optimal control of a stirred tank reactor using Kalman filtering for state estimation", AIChE Journal,**21**, p799, (1975).

Nyquist, J.K. and Ramirez, W.F., "Time optimal control of an experimental continuous stirred tank reactor", Chemical Engineering Science, **26**, p1673-1688, (1971).

Ogata, K., "State space analysis of control systems", Prentice-Hall Inc., Englewoods cliff, N.J., USA, (1967).

Ramirez, W.F., "Process Control and Identification", Academic Press, Boston, USA(1994).

Ray, W.H., "Advanced Process Control", McGraw-Hill Chemical Engineering Series, (1981).

Shah, S.L., Fisher, D.G. and Seborg, D.E., "Design and experimental evaluation of controllers for Process undisturbabilty", AIChE Journal, **27**, p131, (1981)

Waterloo Maple Software, 450 Phillips St., Waterloo, Ontario, Canada N2L 5J2.

3.7 Nomenclature

Roman

b	cumulative biomass in mass units (case study 1)
f	nonlinear function in the process description
k_1	Yield coefficient of CO_2 evolution over biomass
m_c	maintenance coefficient (mmol CO_2 /hr-l)
t	continuous time variable
t_m	time elapsed due to measurement delay
v	measurement noise vector
w	white noise vector

Chapter 3: Structural Observability issues.

x	vector of system states
z	vector of system measurements
A	state transition matrix for the linearized system
C	concentrations in the chemical reactor (case study 2)
F	flow rates of reactants
G	measurement matrix for the linearized system
H	measurement matrix for the linear system
Q	process noise covariance matrix
R	reaction rate in case study 2
R_z	measurement noise covariance matrix
T	temperature of the reactor contents
X	superstate of system and delayed states

Greek

Γ	discrete system matrix
$\delta(.)$	deviation variable
$\partial(.)$	partial derivative
η	measurement noise vector
μ	specific growth rate
ξ	process noise vector
τ	sampling time
Φ	discrete system matrix

Subscript

d	pertaining to the discrete model
d,m	pertaining to the discrete model with modified measurement set
major	pertaining to the major sampling instant
minor	pertaining to the minor sampling instant
o	pertaining to the inlet concentrations
s	pertaining to the steady state around which linearization is done

Chapter 3: Structural Observability issues.

z	pertaining to the measurement vector
KI	pertaining to the catalyst concentration

Acronym

CER	carbon dioxide evolution rate

Chapter 4.

The Role of Adaptive Multirate Kalman Filter as a Software Sensor and its Application to a Bioreactor[1]

A novel approach to address the problem of simultaneous state and parameter estimation for a bioprocess is proposed. The formulation is based on the multirate form of the classical Kalman filter to do model based filtering when multiple rates of measurement availability are present. Model adaptation is performed sequentially using the maximum likelihood based estimation algorithm. The proposed formulation ensures that the task of adaptive estimation is performed using all of and the most up to date measurements. Validation results of the resulting software sensor using experimental data from two different fermentations involving the microorganisms (i) *Saccharomyces cerevisae* and (ii) *Streptomyces clavuligerus* is presented in this chapter.

---

[1] The experimental validation results shown in this chapter were published as :

(1) R.D. Gudi and S.L. Shah, "The Role of Adaptive Multirate Kalman filter as a software sensor and its application to a Bioreactor", *Proc. of 12th IFAC World Congress*, Sydney, Australia, 1993.

(2) R.D. Gudi, M.R. Gray and S.L. Shah, "Multirate Estimation and Monitoring of Process variables in a Bioreactor", *Proc. of 2nd IEEE Conf. on Control Applications*, Vancouver, Canada,1993.

4.1 Introduction

Feedback control requires measured values of variables that need to be regulated . The benefits of feedback control, continuous monitoring and optimization are difficult to realize in a bio-process due to the lack of regular on-line measurements of key variables such as the biomass, substrate concentrations and other secondary products of metabolism. Furthermore, the process has characteristics which vary with time in a non-linear way. Thus a fixed linear model cannot be used in the model-based predictive control algorithms that have proven to be so useful in the chemical industry.

Techniques to overcome the above problems have been used in the past. These include correlating the biomass experimentally with the carbon-dioxide evolution rate using elemental cell balances. Mou and Cooney(1983) have utilized individual elemental cell balances to estimate the time varying yield coefficients and other state variables from measurements of carbon dioxide evolution rate. To account for time varying nature of process and noisy measurements, Stephanopoulos and San(1984) used Extended Kalman filter techniques to estimate the states and parameters from other secondary measurements such as carbon dioxide evolution rate and oxygen uptake rate. Ramirez (1987) has used the sequential parameter updating strategy of Ljung and Soderstrom (1983) to deal with the problem of simultaneous estimation of state variables and model parameters and has successfully used it to regulate nutrient levels in a bioreactor (Park and Ramirez, 1990). Chattaway and Stephanopoulos (1989) have also used the method of sequential parameter updating to monitor plasmid stability for fermentation processes. The overall approach in bioprocess system identification thus has been to use a model, possibly in an adaptive framework, to generate optimal estimates of the states from measurements of all or a subset of all the states.

In a bio-process the states of secondary importance such as carbon dioxide and oxygen contents in exit gas are measurable at a fairly rapid and regular rate. With the advent of on-line flow injection sampling and analysis systems (Reda and Omstead,1990) for infrequent "off-line" monitoring of the bioprocess , the measurements of the key variables such as biomass and the substrate can be made available although only at slower sampling rates. It thus seems logical to use these infrequently available primary measurements as feedback information to the estimator and correct the frequent estimates that are generated inferentially from the frequently available secondary measurements. To achieve this objective, multirate system identification techniques need to be used so as to accommodate multiple rates of sampling that exist in such a scenario. Such techniques also have useful application in traditional control problems such as distillation columns and chemical reactors.

Guilandoust *et al.*(1987,1988) proposed a multirate adaptive inferential estimation algorithm in state space and input-output form. However the inferential relationship they propose is not very evident from their working equations. Lu and Fisher (1990) have formulated the multi-rate estimation problem in such a way that the working equations reflect fully and more fundamentally the inferential relationships. Tham *et al.* (1990) have used an adaptive inferential estimation algorithm in a multi-rate framework to estimate process variables in various processes such as polymerization, distillation columns and continuous fermentors. All of the above approaches to the problem of multirate identification carry out the adaptation on a transfer function model. While such a black box approach has inherent advantages with respect to global applicability, it is useful to study the parameter adaptation to a state space model that results from using a qualitative description of the bioprocess. Ramseir *et al.*(15) have developed an adaptive scheme to estimate culture states from CER and base addition rate measurements for a continuous yeast fermentation. Stone *et al.*(19) have used off-line measurements to update the changing maintenance coefficient, using a balancing method of Esener *et al.* (1), during the secondary production phase in an antibiotic fermentation. Lee and Morari(1992) have established a generalized inferential control framework for designing robust linear controllers for multi-rate systems.

In this chapter, a new approach to the problem of adaptive multirate filtering and estimation is presented. To generate optimally filtered state estimates conditioned on the best available model when the measurements arrive at different sampling rates, model based multirate Kalman filtering (MKF) technique of Glasson (1980,1983) is used. The model that is needed for the MKF based filtering is adapted to the time varying characteristics of the process using the maximum likelihood based sequential parameter updating strategy of Ljung and Soderstrom(1983). The proposed strategy is experimentally evaluated by application to two different fed batch fermentations.

4.2 Process Model

In this chapter, the following case studies have been considered for estimator validation:

(i) growth of baker's yeast (*Saccharomyces cerevisae*) on glucose in a fed batch fermentation, and

(ii) Fed batch fermentation of *Streptomyces clavuligerus* NRRL 3885 in a complex media.

The material balance equations describing the growth of baker's yeast on glucose can be written as follows:

$$\frac{d(x)}{dt} = \mu\ (x) \tag{4.1}$$

$$\frac{dC}{dt} = F_g\ C_{in} - k_1\mu(xV) - F_g\ C \tag{4.2}$$

$$\frac{dG}{dt} = k_2\mu(xV) - F_g\ G \tag{4.3}$$

where

x is the cumulative biomass in (g); C and G are the volume fractions of oxygen and carbon-dioxide in the exit gas; μ is the specific growth rate of the biomass; k_1,and k_2 are functions of appropriate yield coefficients; F_g is a variable that depends on the gas phase dynamics and is assumed to be approximately constant; C_{in} is the inlet concentration of oxygen in the sparged gas and the glucose concentration in the feed and V is the broth volume.

The above equations can be discretized using a simple euler discretization to yield the following discrete model,

$$X(t+1) = A(\theta,\tau)\, X(t) + B\, u(t) + \eta_x \,;\; E(\eta_X\, \eta_X^T) = R_X \tag{4.4}$$

where

$$X(t) = [(x)_t,\, C_t,\, G_t] \tag{4.5}$$

and η_x is the discretization error. The concentrations of oxygen and carbon dioxide in the exit gas constitute the vector of measured system outputs. R_Z is assumed to be the covariance matrix of measurement noise for these system outputs.

The second case study involves the microorganism *Streptomyces clavuligerus* growing in a complex medium. The fermentation exhibits endogenous metabolism, changing maintenance activity and secondary product (Antibiotic) expression during the fermentation. The system state (biomass) dynamics can be written after performing a simple euler discretization of the dynamic equation for growth as,

$$x(i+1) = [1+\tau\mu(i)]x(i) + w(i) \;;\; E(ww^T) = R_x \tag{4.6}$$

Assuming that the amount of CO_2 in the broth is approximately constant, the system output equation can be written in terms of the carbon dioxide evolution rate(CER) as,

$$CER(i) = [k_c\mu(i) + m_c(i)]x(i) + v(i);\; E(vv^T)=R_z \tag{4.7}$$

k_c is the yield coefficient relating biomass growth to CER and m_c is the maintenance coefficient. The CER can be directly calculated online from measurements of CO_2 content in the exit gas.

Measurements of the variables such as CO_2 and O_2 contents in the exit gas are available at a fairly fast sampling rate. However these provide only indirect information about the state of fermentation and hence are termed secondary variables. Measurements of the variables such as biomass and substrate are available only at a slower sampling rate. Since these are of direct relevance, they are termed the primary variables. τ is the sampling time with respect to the secondary variables and we assume that the primary measurements are available every $J\tau$ time units ($J > 1$). At instants when primary measurements are available, the secondary ones are also available and we term this sampling instant as a major sampling instant. At all other sampling instants , only the secondary measurements are available and these are termed as minor sampling instants.

4.3 Adaptive Multirate Kalman filter -a unified approach

For systems with unknown parameters or for time-varying systems, there are two basic approaches to constructing a state estimator (Goodwin and Sin,1984). The first is the extended Kalman filter approach in which the state vector is augmented by addition of the unknown parameters. The unknown parameters are then identified using information available as innovations. However, this approach is known to give divergence problems (Ljung,1979). The recommended approach is to use the fact that the Kalman filter for linear systems actually coincides with a one-step ahead predictor. The prediction error resulting from the Kalman filter can then be used to estimate the parameters using a bootstrap method.

Park and Ramirez(1990) have illustrated the latter approach using the concepts of conditional probability density.

4.3.1 Multi-rate Kalman Filter

We first consider the maximization of the probability density function for state estimation conditioned upon uncertain process parameters. The Kalman filter gives an optimal estimate of the states conditioned on the best parameter estimate available(Jazwinski,1970). Since multiple rates of sampling exist, the classical Kalman filter needs to be expressed in multi-rate form to accommodate them. In this multirate formulation(Glasson,1980), the dimension of the measurement vector , measurement noise covariance and the Kalman filter gains are assumed to vary periodically between the major and minor sampling instants.

At the major sampling instant, the measurements of all the states are available and therefore the measurement vector H_{major} is the identity matrix for the said model and the measurement equation can be written as

$$z_{major} = H_{major} X \tag{4.8}$$

The measurement noise covariance matrix corresponding to the major sampling instant would then be a larger diagonal matrix of measurement noise covariances.

The Kalman gains can then be evaluated at the major sampling instant as,

$$K_{major} = M\ H_{major}\ (H_{major}\ M\ H^{T}_{major} + R_{z,major})^{-1} \tag{4.9}$$

where M is the apriori estimate of the covariance matrix. Note that the Kalman gain matrix is also a square matrix of size equal to the number of states.

At the minor sampling instants, only the secondary measurements are available and therefore the measurement vector H_{minor} is a nonsquare matrix with as many rows as the number of measurements and the measurement equation can be written as,

$$z_{minor} = H_{minor} X \tag{4.10}$$

The measurement noise covariance matrix $R_{z,minor}$ corresponding to the minor sampling instants would then be a smaller diagonal matrix of measurement noise covariances.The Kalman gains for the minor sampling instant can then be written as

$$K_{minor} = M\ H^{T}_{minor}\ (H_{minor}\ M\ H^{T}_{minor} + R_{z,minor})^{-1} \tag{4.11}$$

Note that for the minor sampling instant, the Kalman gain matrix is a nonsquare matrix with as many columns as the number of measurements.The overall Kalman filter equations in multirate form would then be the same as the those of the classical Kalman filter, but with periodicity in Kalman gains,measurement and process covariance matrices. The two step formulation of the time-varying Kalman filter (Franklin *et al.*,1990) can then be used to express the Kalman filter in its multi-rate form as :

a) Major sampling instant :

i) Measurement update:

$$\hat{X}(i) = \overline{X}(i) + K_{major}\ \varepsilon(i) \tag{4.12}$$

$$\varepsilon(i) = z_i - H_{major}\ \overline{X}(i) \tag{4.13}$$

$$P(i) = M(i) - K_{major} H_{major} M(i) \tag{4.14}$$

ii) Time update :

The time update equations can be written as,

$$\overline{X}(i+1) = A\ \hat{X}(i) + B\ u(i) \tag{4.15}$$

$$M(i+1) = A\ P(i)\ A^T + Q \tag{4.16}$$

where Q is the covariance of the model uncertainty.

b) Minor sampling instant :

i)Measurement update:

$$\hat{X}(i) = \overline{X}(i) + K_{minor}\ \varepsilon(i) \tag{4.17}$$

$$\varepsilon(i) = z_i - H_{minor}\ X(i) \tag{4.18}$$

and the Kalman gain matrix is given by (4.11).

$$P(i) = M(i) - K_{minor}\ H_{minor}\ M(i) \tag{4.19}$$

ii)Time update :

The time update equations can be written as,

$$\overline{X}(i+1) = A\ \hat{X}(i) + B\ u(i) \tag{4.20}$$

$$M(i+1) = A\ P(i)\ A^T + Q \tag{4.21}$$

4.3.2 Model estimation

To generate optimal state estimates as shown in the previous section, we need to have a fairly accurate idea of the signal and noise covariances which reflects in the evaluation of the Kalman gain matrix. The latter depends on the ratio of the process noise covariance to the measurement noise covariance. A high ratio of these covariances would mean that the measurements are weighted more heavily in the generation of the optimal estimates than the process model. However, at the minor sampling instants, measurements for the primary variable do not exist and therefore a high value of the covariance ratio would deteriorate the estimates of the primary process variable. At low ratios of these covariances, the model plays a key role in the generation of the optimal estimates through the time update equation and therefore one needs to have a model with a good prediction capability to perform the time update. Identifying such a model is equivalent to maximizing the probability function for parameter estimation i.e. identifying the parameter vector θ that gives the best prediction capability at any instant i. A general objective function to identify θ has been proposed to be of the form(Goodwin and Sin,1984),

$$V_N(\theta) = \frac{1}{N}\sum l(\hat{y}(i,\theta)y(i)) \tag{4.22}$$

where $\hat{y}(i,\theta)$ is the prediction conditioned on the best available estimate of θ and y(i) is the measurement. They have also shown it to be statistically related to the maximum likelihood estimation of θ. A typical choice of the objective function could be

$$V_N(\theta) = \frac{1}{2}\sum \varepsilon^T(i,\theta)\Lambda^{-1}\varepsilon(i,\theta) \tag{4.23}$$

where Λ is a positive definite weighting matrix.

A recursive maximum likelihood algorithm to minimize the above objective function is provided by Ljung and Soderstrom(1983) and can be summarized as follows :

$$\hat{\theta}(i) = \hat{\theta}(i-1) - H^{-1}(\hat{\theta}(i-1))\; J(\hat{\theta}(i-1)) \tag{4.24}$$

where J and H_s are the gradient and the hessian of the objective function respectively and are updated using the following recursive relationships :

$$J(\hat{\theta}(i-1)) = -\psi(i)\; \Lambda^{-1}\; \varepsilon(i) \tag{4.25}$$

$$H_s(\hat{\theta}(i-1)) = H_s(\hat{\theta}(i-2)) + \psi(t)\Lambda^{-1}\psi(i)^T \tag{4.26}$$

where ψ is the negative gradient of prediction error $\varepsilon(t)$ with respect to θ and is evaluated using a recursive relationship (Ljung and Soderstrom,(1983)), (Park and Ramirez (1990)). The weighting matrix Λ must be equal to the covariance of prediction errors(Ljung and Soderstrom,1983). Since an initial estimate of this is not known, it is updated using the recursive relationship,

$$\Lambda(i) = \Lambda(i-1) + \gamma(i)(\boldsymbol{\epsilon}(i)\boldsymbol{\epsilon}^T - \Lambda(i-1)) \tag{4.27}$$

where γ is a suitably chosen gain sequence. The bootstrap strategy presented in the above sections can then be said to be optimal with respect to the maximum likelihood criterion. As is evident from the multirate Kalman filter equations presented in section 3.1, the innovations in both the primary and secondary variables are used to generate optimal state estimates of both the variables. Further, the sequential parameter updating strategy presented in this chapter uses information on the prediction error at every instant to update the process model matrix A. The proposed strategy thus generates optimal estimates of all the states and the model parameters at every sampling instant.

Remarks:

- From an implementation point of view, the observability aspects of the states and parameters need to carefully considered. A symbolic observability analysis carried out for the system considered in the first case study (fed batch fermentation of Baker's yeast) shows the system to be fully observable at the major as well as the minor sampling instants. For the antibiotic producing fermentation of *Streptomyces clavuligerus*, the overall system was found to be partially observable at the minor sampling instants. Thus, only a subset of the overall system viz. the state (biomass) and one of the parameters (specific growth rate μ) could be updated from the innovation resulting from the CER measurement. The system was found to be fully observable at the major sampling instants. So the maintenance coefficient parameter was updated only at the major sampling instant.
- Another implementation aspect that needs attention is the efficiency of the recursive update algorithm for the updating of parameters of a time varying system. Kumar and Moore (1980) have proposed a gain sequence to keep the updating algorithm sufficiently alert as follows :

$$\gamma_k^{-1} = 1 + K_g \gamma_{k-1}^{-1} \tag{4.28}$$

where γ is initialized by a value greater than 1-K_g. The gain sequence converges to a final value determined by the choice of K_g which can be chosen depending on the time-varying characteristics of the process. This has been used in the above updating step to keep the algorithm alert. The updating step also needs inversion of the hessian matrix H. This matrix suitably modifies the gradient search direction to a more efficient one. For the recursive case, the updating of this hessian matrix depends on several factors such as the choice of the gain sequence and may not accurately represent the hessian of the objective function proposed. Besides, the inversion step itself could be prohibitive. However, to ensure that minimization of the proposed objective function occurs, any positive definite matrix could be used in place of the hessian and the stochastic gradient algorithm is a direct result of using such an implementation. In this algorithm, the choice of the hessian is made to be some multiple of the identity matrix and the scaling is chosen to be the trace of the matrix $\Psi\Lambda^{-1}\Psi$. In the model updating step of the proposed multirate algorithm, the gain sequence described above coupled with the stochastic gradient algorithm has been used to perform the task of parameter estimation.

4.4 Experimental evaluation

4.4.1 Experimental system I : Fed batch Fermentation of *Saccharomyces cerevisae*

The strategy was evaluated on experimental data generated from a fed-batch fermentation of *Saccharomyces Cerevisiae* by Namdev(1991). Fed batch fermentation with an exponentially increasing feed strategy was started from chemostat conditions established in a culture of *S.Cerevisiae*. The feed concentration of glucose was 100 g/l and was supplied to the culture at an exponential rate according to,

$$F(t) = \frac{\mu\ V_o X_o}{Y_x S_{in}}\ e^{\mu t}$$

where the initial conditions used were: biomass X_o = 2.5g/l; fermentor volume V_o=1L, glucose concentration in feed S_{in}=100 g/l. The desired growth rate μ was assumed to be 0.16 hr^{-1} and the biomass yield on substrate Y_x was assumed to be 0.45.

The fermentor off-gas was analyzed for O_2 and CO_2 by a Dycor quadruple mass spectrometer every two minutes. Infrequent off-line assays of the biomass was available from the experimental data. However these were irregular and spaced at 1.5 hour intervals. It was thus decided to use a model developed for fed-batch fermentation based on the hypothesis of Sonnleitner and Kappeli(1986). The model parameters proposed by the above authors were adjusted to give good match of (i) the simulated profiles of the biomass with the infrequent experimental assays and (ii) the simulated exit gas concentrations with those obtained experimentally.

The strategy was thus evaluated by using

(i) frequent measurements of exit gas concentrations from the experimental data every 6 minutes and

(ii) infrequent but regular measurements of the biomass along with culture volume from the model simulations every 18 minutes. Random noise to the extent of 10 % of the measured values was added to simulate noisy measurements.

4.4.1.1 : Results and discussions

The success of the multi-rate adaptive estimator depends among other things on its ability to generate optimal estimates of the primary variables at minor sampling instants from secondary innovations. Obviously, the ratio of covariances of states and measurements is an important tuning knob for the performance of the filter-estimator. For the case when all the states are measured regularly, Park and Ramirez(1992) have shown this to be true. A high value of this ratio would give less of model-based filtering action but the parameter adaptation is faster. A low value would give optimally filtered estimates,especially at the minor sampling instants for the primary variable, but the adaptation of the model would be slow. For our estimation, the optimal covariance matrices were Q=diag(5,5,5) and R_z=diag(1,10,1).

Figure 4.1 shows the periodicity in the elements of the MKF. At minor sampling instants, the primary states are estimated from the optimal estimates of the secondary states and the secondary innovations. The error covariance and the off diagonal Kalman gains (Kalman gains of the primary estimate from the secondary innovation) increase in size till the next major sampling instant. At this point, an accurate estimate of the primary variable is generated due to the primary variable itself and therefore the cross Kalman gains and the error covariance are reset. This behaviour is periodic between major sampling instant. Figure 4.1 is plotted to show this periodic behaviour by assuming that the major sampling instant arrives after every 6 minor sampling instants. Thus after the 12th sampling instant which is a major sampling instant, the trace of the covariance of errors and the cross Kalman gains rise till the 18th sampling instant which is the next major sampling instant, when they both are reset to low values. Figure 4.2 shows the adaptation of the critical parameter θ_1 which is associated with the biomass growth. For a predicted specific growth rate of 0.16 hr^{-1} and sampling time of 0.1 hr, the expected value of θ_1 is 1.016. As can be seen from this figure, the parameter estimate converges quite rapidly to its expected value of 1.016.

Figure 4.3 shows the comparison of the MKF state (biomass) estimates with those available experimentally. It can be seen that MKF in association with the parameter estimator gives good estimates of all the states from noisy measurements. True filtering action is clearly observed for the oxygen content in exit gas in Figure 4.4. Since this measurement has a lot of noise associated with it, the measurement noise covariance for this state is specified to be greater than the state covariance. Thus the measurements are weighted to a lesser extent in the state estimate and the Kalman gains are small. The smaller filter gains affect the sensitivity of the state estimator as can be observed towards the end of the fermentation run when the oxygen uptake rate drops resulting in higher oxygen contents in the exit gas. This behaviour is tracked slowly by the state estimator.

This slow tracking is also due to the slower model adaptation for the parameter $\theta(2)$ due to the low Kalman gain associated with this measurement. For all other variables, the measurement noise is small and therefore the measurement noise covariance is specified to be small. Thus the measurements get higher weighting in the state estimates. Excellent match of the experimental data is observed for the last state variable viz., CO_2 content in the exit gas.

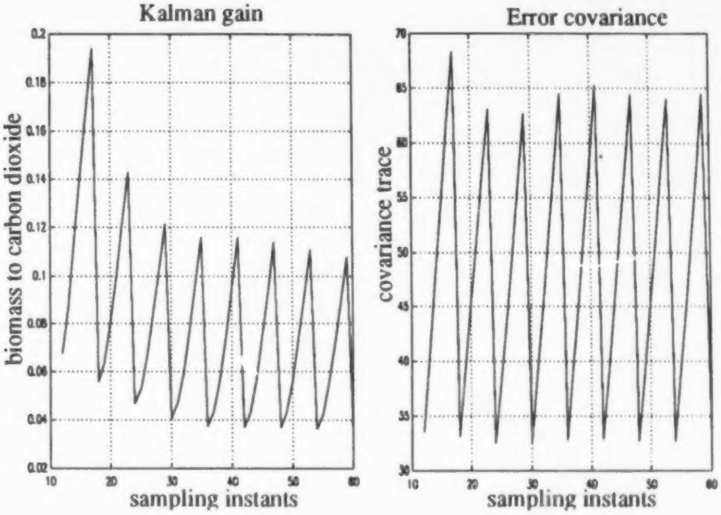


Figure 4.1 Periodicity in the multirate Kalman filter (MKF) elements : The Kalman gains and the error covariances increase at the minor sampling instants and are reset at the major sampling instant.

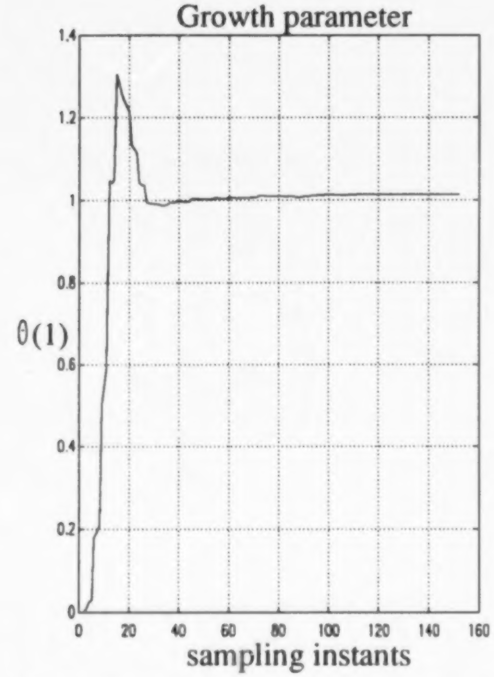


Figure 4.2 Profile of an estimated parameter for the fed-batch fermentation of *Saccharomyces cerevisae*. The growth rate related parameter converges to its expected value of 1.016.

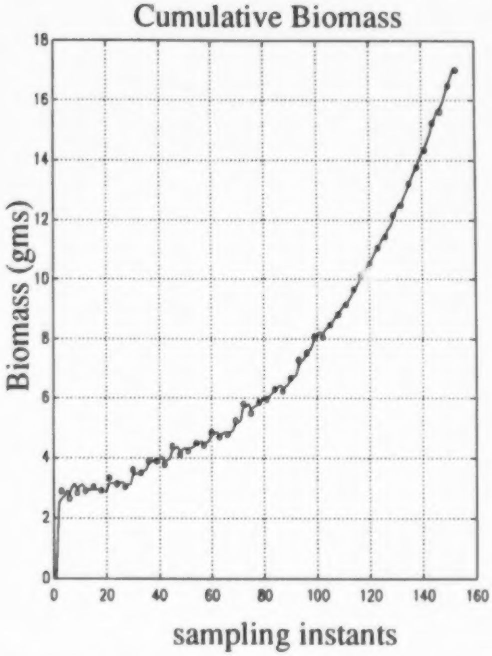
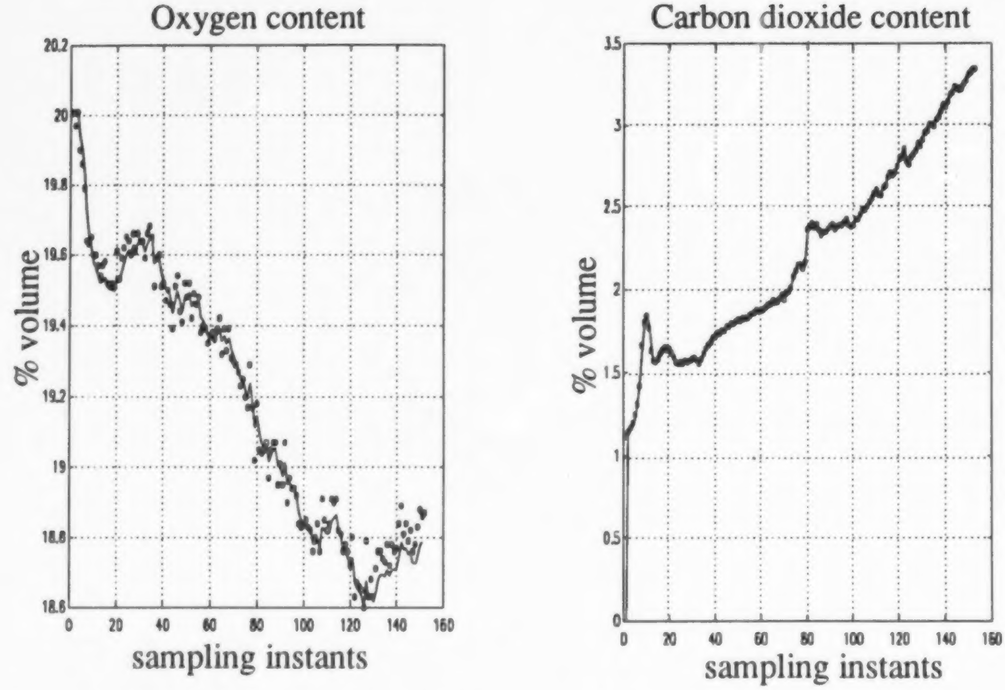


Figure 4.3 State estimate profiles using the MKF for the Saccharomyces system: Excellent agreement is seen between the estimated and measured values.


Oxygen content

Carbon dioxide content

Figure 4.4 Exit gas profiles : MKF based filtering of the exit gas oxygen measurements for the saccharomyces system.

4.4.2 Experimental system II : Fed batch Fermentation of *Streptomyces clavuligerus*

Experimental data from fed batch fermentation of *Streptomyces clavuligerus* NRRL 3885 in complex media was used to analyze the performance of the algorithm. The fermentation exhibits a lysis phase after about 24 hours of fermentation in the fed-batch mode and so a feeding strategy as shown in Figure 4.5 was chosen to minimize substrate limitation of the secondary product (Antibiotics).

It was decided to study the monitoring of biomass using (i) CER measurements available every 20 minutes and (ii) Biomass measurements available every 3 hours. A two parameter model relating CER to the growth and maintenance associated activities of the culture was used as an inferential model. The CER measurements were generated by monitoring exit gas concentrations using a Dycor mass spectrometer and the biomass was monitored by optical density measurements.

4.4.2.1 Results and Discussion

Secondary product expression takes place typically at low growth rates. During this phase of the fermentation, the maintenance activity and endogenous metabolism in the culture is expected to be significant(Mou *et al.* (1983)). It is therefore necessary to express the overall CER as the sum of its growth associated and maintenance associated terms and update the value of the maintenance coefficient, whenever possible, to prevent bias in the estimation of the biomass and the specific growth rate. Stone *et al.* (1992) performed the updates of the maintenance coefficient by using the balancing method of Esener *et al.*(1981). This method is however accurate only if the fractional increase in biomass is small and has thus been used for updation only during the production phase. The adaptive multirate estimation algorithm proposed in this chapter does not have the above restriction. It can therefore be used in a more general way even when the maintenance coefficient is not accurately known.

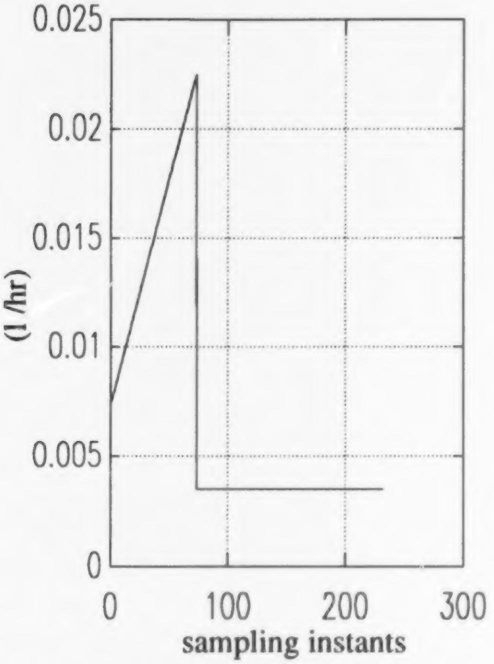


Figure 4.5 The nutrient feeding profile for the antibiotic fermentation. The nutrient feeding profile shown above was used to prevent substrate inhibition of growth and product expression.

Since the Kalman filter in a multirate framework forms the basis of this algorithm, the incorporation of the infrequently available measurement into the overall optimal filtering and estimation strategy is also more formal.

Figure 4.6 shows the estimation of the biomass. The tracking of biomass in the initial linear region of growth is quite good. At the major sampling instants a measurement of the biomass becomes available and this is used to correct the estimates derived inferentially from CER measurements. These corrections are seen as small spikes in the estimated biomass profile. During the period of lower growth and significant maintenance activity, these corrections are quite drastic.This is because the maintenance coefficient changes with time and the inferential model relating CER to growth alone is not capable of generating estimates of the growth rate and biomass. It is therefore necessary to incorporate a two parameter model relating CER to growth and maintenance associated activities of the culture and track the changing maintenance coefficient whenever possible. Figures 4.7 and 4.8 show the estimates of the specific growth rate μ and the maintenance coefficient m_c respectively. The algorithm was initialised with values for μ and m_c equal to 0.25 hr^{-1} and 1.1 mmol CO_2/hr-g biomass respectively. It can be seen that the tracking of the specific growth rate and the maintenance coefficient is excellent in the region of linearly increasing feed strategy. Antibiotics are expressed after about 8 hours of growth in the fed batch mode or at about the 25th sampling instant. At about the 75th sampling instant, the feed is lowered to a constant value. This also approximately coincides with the lysis phase observed in the fermentation. The estimation of the growth rate and the maintenance coefficient is as expected. The growth rate drops to zero and then becomes negative to indicate cell lysis and then returns to a small constant value around zero. The maintenance coefficient increases to a value close to 1.8 mmol CO_2 /hr-g biomass to indicate an increase in the maintenance activity of the culture. It is easy to see that this is the also the value that would be predicted from the terminal conditions of CER and the total biomass weight .

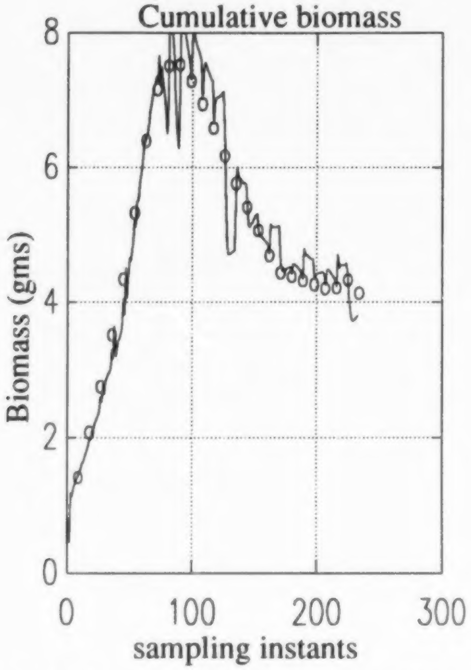


Figure 4.6 MKF state estimates for the antibiotic fermentation: The cumulative biomass shows good agreement with the experimental values.

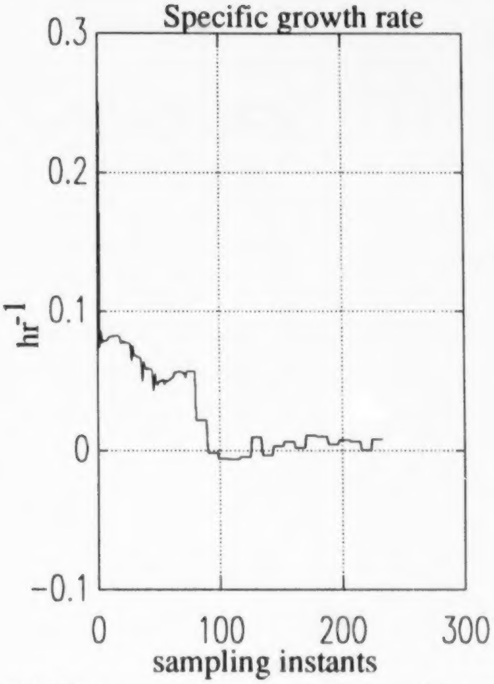


Figure 4.7 MKF based estimation of the specific growth for the antibiotic fermentation.

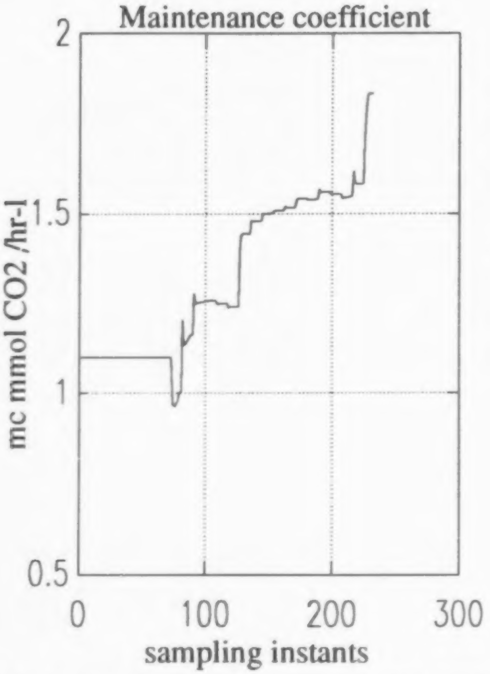


Figure 4.8 MKF based estimation of the maintenance coefficient for the antibiotic fermentation.

Since the growth rate is a small positive value close to zero at the terminal point, all the CO_2 evolved can be attributed to maintenance activity in the culture. Knowing the terminal biomass weight enables evaluation of the maintenance coefficient to be about 1.9 mmol CO_2 /hr-g biomass. This is close to the estimated terminal value of the maintenance coefficient. The algorithm is thus capable of generating regular estimates of the primary state viz. the biomass and the parameters μ and m_c.

4.5 Conclusions

The multirate adaptive estimator derived above has been shown to have a good theoretical basis. It is based on decomposing the problem of simultaneous identification of states and parameters. Optimal estimates of the states , conditioned on the best available model, are generated using the multirate formulation of the classical Kalman filter. The model used in the filtering is adapted using a sequential updating strategy. The multirate adaptive estimator thus utilizes information in both the primary and secondary measurements in an optimal way to generate regular, filtered estimates of all the states from

(i)frequent measurements of the secondary states ;

(ii)infrequent measurements of the primary states.

The performance of the multirate adaptive estimator has been experimentally evaluated by application to two different fed batch fermentations.

4.6 References

Franklin, G.F and Powell, J.D, "Digital Control of Dynamic systems", Addison-Wesley Publishing Co., Reading, Massachussets, USA(1980).

Glasson, D.P, " Research in Multirate estimation and control", Analytic Sciences Corporation, Six Jacob Way, Reading, Massachussets, USA (1980).

Glasson, D.P, "Development and Applications of Multirate Digital Control ", IEEE Control Systems Magazine, 3(4), p2-8, (1983).

Goodwin, G.C and Sin, K.S., " Adaptive Filtering, Prediction and Control", Prentice Hall Inc., Englewoods Cliffs, New Jersey, USA (1984).

Guilandoust, M.T., Morris, A.J. and Tham, M.T., "An adaptive estimation algorithm for Inferential Control", I.E.C Research, **27**, p1658-1664, 1988.

Guilandoust, M.T., Morris, A.J. and Tham, M.T., " Adaptive Inferential Control", IEE Proceedings, Part D, **134**(3), p171-179, (1987).

Jazwinski, A.H., "Stochastic Processes and Filtering Theory ", Academic Press,New York, USA (1970).

Kumar, R. and Moore, J.B., "State inverse and decorrelated state stochastic approximation", Automatica, **16**, p295-311, (1980).

Lee, J.H and Morari, M., "Robust Inferential Control of Multirate sampled data systems", Chemical Engineering Science, **42**, p865-885,(1992).

Ljung, L., " Asymptotic behaviour of Extended Kalman Filter as parameter estimator for linear systems", IEEE Trans. Automatic Control, **AC-27**(1), p 36-51, (1979).

Ljung, L and Soderstrom, T., "Theory and Practice of Recursive Identification", MIT Press, Cambridge, USA (1983).

Lu, W. and Fisher, D.G.," Multirate Constrained Adaptive Control", International Journal of Control, **51**, p1439-1456, (1990).

Mou, D.G and Cooney, C.L., " Growth Monitoring and Control through Computer-aided on-line mass balancing in a fed-batch Penicillin fermentation", Biotechnology and Bioengineering, **25**, p225-255, (1983).

Namdev, P.K., " Nutrient fluctuations in fed-batch fermentation of Baker's Yeast", Ph.D thesis, University of Alberta, Edmonton, Canada,(1991).

Park, S. and Ramirez, W.F., "Optimal regulatory control of bioreactor nutrient concentration incorporating system identification", Chemical Engineering Science, **45**(12), p3467-3481, (1990).

Ramirez, W.F., "Optimal state and parameter Identification : an application to batch fermentation", Chemical Engineering Science, **42**(11), p2749-2756, (1987).

Reda, K.D and Omstead, D.R., " Automatic fermentor sampling and stream analysis", I " Computer Control of Fermentation Processes", Ed. D.R. Omstead, CRC Press, Boca Raton, Florida, USA(1990).

Sonnleitner, B. and Kappeli, O., " Growth of *Saccharomyces Cerevisiae* is controlled by its limited respiratory capacity: Formulation and verification of a Hypothesis",Biotechnology and Bioengineering, **28**, p927-937,(1986).

Stephanopoulos, G. and San, K.Y.," Studies on on-line bioreactor identification", Biotechnology and Bioengineering, **26**, p1176-1188, (1984).

Tham, M.T., Montague, C.A., Morris, A.J., Lant, P.A., " Soft sensors for process estimation and inferential control", Journal of Process Control, **1**, p3-14, 1991.

4.7 Nomenclature

Roman

k_1,k_2	functions of appropriate yield coefficients.
k_c	yield constant for CO_2 evolution
m_c	maintenance coefficient in mmol CO_2/l-hr
u	control input to the system
w	white noise vector
x	cumulative biomass in mass units
z	vector of measurements at any instant i
A,B	discrete state transition matrices
C	volume fraction of oxygen in exit gas
F	variable dependent on gas phase dynamics
G	volume fraction of carbon dioxide in the exit gas
H	measurement matrix at any sampling instant
H_s	hessian of the maximum likelihood objective function
J	gradient of the maximum likelihood objective function
K	Kalman gain matrix at any sampling instant
K_g	constant for gain sequencing
M	*a priori* estimate of the covariance of estimation errors.
P	*a posteriori* estimate of the covariance of estimation errors.

Q	covariance matrix of process noise
R	covariance matrix of measurement noise
V	maximum likelihood objective function
X	system state vector for the first fermentation

Greek

γ	gain sequence for the weighting matrix
ε	innovations vector at any sampling instant
η	process noise including errors due to discretization
θ	vector of system parameters
Λ	weighting matrix for the maximum likelihood function
μ	specific growth rate for the microorganisms
τ	sampling time

Superscripts

^	state estimate after measurement
-	state prediction via time update equation

Subscripts

g	relating to gas phase dynamics
in	pertaining to the inlet conditions
z	associated with the measurement vector in second case study
Z	associated with the measurement vector in the first case study
x	related to the process in the 2nd case study
major	pertaining to the major sampling instant
minor	pertaining to the minor sampling instant

Acronym

CER	carbon dioxide evolution rate

Chapter 5

Development of a multirate EKF based state and parameter estimator[1]

This chapter is concerned with the design and development of a multirate software sensor for use in the chemical process industry. The measurements of process outputs that arrive at different sampling rates are formally accommodated into the estimation strategy by using the multirate formulation of the iterated extended Kalman filter. Measurement delays associated with some of the process outputs are included in the system description by addition of delayed states. Observability issues associated with state and parameter estimation in a multirate framework are discussed in detail and modified measurement equations are proposed, for systems with delayed measurements, to ensure relatively "strong" system observability. The evaluation of the proposed multirate state and parameter estimator through simulations on a fed batch fermentation system gave satisfactory performance and illustrated the practicality of this approach.

---

[1] A version of this chapter has been accepted for publication in the AIChE Journal as : R.D. Gudi, S.L. Shah and M.R. Gray, "Adaptive Multirate State and Parameter estimation strategies with application to a bioreactor".

Chapter 5: Development of a multirate EKF based estimator.

5.1 Introduction

This chapter addresses issues related to continued monitoring of process outputs when they are measured at infrequent and/or irregular times with or without a delay. Such problems are frequently encountered in typical chemical and biochemical processes when it is often difficult to measure the key process variables on-line on a regular basis due to lack of adequate sensors. In addition. even if such measurements are possible, there may be delays between the time the samples are taken and when the actual measurements are obtained due to elaborate assay procedures. Typical examples of traditional chemical processes where such problems arise are fermentation reactors, distillation columns and polymerization processes. In particular, this study examines the issues of software sensors or inferential estimators in the context of classical control concepts of system observability and Kalman filters based on state-space system descriptions.

Inferential estimation strategies have been and continue to be used to address the above problems. An inferential estimation strategy uses frequently available measurements of secondary process outputs whose behaviour is correlated with that of primary output (process variables or outputs that need to be carefully monitored and or controlled) via a process model that describes the correlation, to generate frequent regular estimates of the primary output. If the process is time-varying, the process model can be estimated and updated in an adaptive framework.

Early work in the area of inferential estimation for chemical processes for the time-invariant case was done by Brosilow and co-workers (Brosilow (1978,1979) and Joseph *et al.* (1978)). Morari *et al.* (1980) have discussed strategies for optimal selection of secondary measurements to perform state estimation in the face of persistent unknown disturbances. Applications in the area of inferential estimation for bioprocesses include the work by Mou *et al.* (1983), Stephanopoulos *et al.* (1984) and Bastin and Dochain(1990). In the study by Mou *et al.* (1983), elemental cell balances based on an empirically derived molecular formula for the biomass formed the inferential model that related secondary outputs such as the carbon dioxide evolution rate (CER) to the biomass (primary output) growth. In the antibiotic fermentation considered by them, a different correlation was used in the production phase to account for the time-varying process behaviour resulting from the changing maintenance activity of the culture. To account for noise in real time measurements and also simultaneously estimate critical parameters of the culture such as specific growth rate and the culture states, Stephanopoulos *et al.* (1984) proposed the extended Kalman filter (EKF) approach. They recommended compensation of the secondary measurements for the maintenance activity if the latter was significant. The EKF is, however, known to be very sensitive to modelling errors and can generate biased estimates of the states in the presence of model plant mismatch. In a different approach to simultaneous state and parameter identification, Ramirez (1987) and Chattaway *et al.* (1989) used the Kalman filter coupled with the sequential parameter updating strategy of Ljung and Soderstrom (1983) to perform the state and parameter estimation. Park and Ramirez (1990) have also successfully applied the above strategy to regulate nutrient levels in a bioreactor. In their experimental setup, however, the primary state variables such as the biomass and substrate concentrations were measurable on an on-line basis.

The performance of inferential estimation strategies proposed above can be made more robust by formally incorporating the infrequently available primary measurement. With the advent and possible application of on-line flow injection sampling and analysis systems (Reda and Omstead, 1990) or by including infrequent "off-line" monitoring of the bioprocess, such measurements can be made available only at slow sampling rates. It thus appears logical to incorporate these infrequently available measurements formally as feedback information to the estimator, i.e. use this information to correct the frequent estimates of the primary states that are generated inferentially from the frequently available secondary measurements. To achieve this objective when the process is time varying, adaptive multirate system identification strategies need to be used to formally accommodate the two or more sampling rates that exist in such a scenario. Guilandoust *et al.*(1987,1988) proposed an adaptive multirate inferential estimation algorithm in state space and transfer function form. Lu and Fisher (1990) have formulated the multirate estimation problem such that the working equations explain the inferential relationships in a more fundamental way and thus have formally proved the convergence properties of such an inferential control algorithm. Tham *et al.*(1990) have used an adaptive inferential estimation algorithm in a multirate framework to estimate process variables in various processes such as polymerization reactors, distillation columns and continuous fermentors. All of the above approaches to the problem of multirate identification carry out the adaptation of a transfer function model of the process. While such a black box approach has inherent advantages with respect to global applicability, it is also useful to study the parameter adaptation of a state space model that results from a qualitative or structural description of the process. The merits of the latter lie in the use of *apriori* information such as mass and energy balances, albeit in a qualitative form, for performing estimation. The model parameters in such a state space description also have a physical significance in relation to the process. Such *apriori* process information is not directly useable in the transfer function based approaches. It is also often difficult to relate transfer function parameters to the physical parameters of the actual process.

The main focus of this chapter is the introduction of a novel approach to address the problem of adaptive multirate filtering and estimation. To generate optimally filtered estimates of the primary states when the measurements arrive at different sampling rates, the model-based multirate Kalman filtering strategy of Glasson(1980,1983) is used. The model that is used is the type that would result from direct linearization and discretization of the mass and energy balance equations based on a qualitative description of the process. To adapt the model used for the inferential estimation to the time varying characteristics of the process, a Bayesian formulation of the multirate Kalman filter is used so as to include estimation of the time varying parameters. The deterioration in estimator performance caused by nonlinearities in the measurement equation is addressed by using the iterative version of the extended Kalman filter (Jazwinski,1970) expressed in a multirate framework. Delays, that result due to relatively long analysis times in elaborate off-line assay procedures, are accounted for in the above formulation by incorporating delayed state variables in the state space model. The incorporation of delayed states increases the order of the model and thus reduces the relative observability of the overall system. Therefore, to enhance system observability, we propose retaining past measurements in the output equations. Thus, a simple yet robust method to ensure relatively strong system observability at all sampling instants has been incorporated. The resulting multirate, iterative, extended Kalman filter based sensor is evaluated by simulated and experimental applications on antibiotic fermentations involving the microorganism *Streptomyces clavuligerus.*

5.2 Problem Formulation

Consider the following state description of the process :

$$\dot{\underline{x}} = f(\underline{x},\underline{u},\underline{\theta},t)+\underline{\xi}(t) \tag{5.1}$$

$$\underline{z} = \underline{g}(\underline{x},\underline{u},\underline{\theta},t) + \underline{\eta}(t) \tag{5.2}$$

where $\underline{\xi}$ and $\underline{\eta}$ are both zero mean, independent noise processes with unspecified distributions. The state vector $\underline{x}$ could consist of states such as reactant/product concentrations in a semi-batch exothermic reactor or biomass/substrate concentrations in a fed-batch bioreactor. In a chemical or a biochemical process, these are the states of primary interest but are, however, sampled slowly, sometimes off-line, due to lack of adequate on-line sensors. We term these state variables as primary variables. A suitable requirement for control is that these measurements be available more frequently. Let the size of the state vector $\underline{x}$ be n. The parameter vector $\underline{\theta}$ contains the time varying system parameters. These parameters are modelled as zero mean white noise processes with unspecified distributions. Thus,

$$\dot{\underline{\theta}} = \underline{w}(t) \tag{5.3}$$

Let the dimension of θ be $n\theta$. Commonly, the time varying parameters θ are appended to the system state $\underline{x}$ to constitute an augmented state vector $\underline{X}=[\underline{x};\theta]$. The augmented system description can be written as,

$$\underline{\dot{X}}=F(\underline{X},\underline{u},t) + \underline{\xi}_1(t) \tag{5.4}$$

$$\underline{z} = G(\underline{X},\underline{u},t) + \underline{\eta}(t) \tag{5.5}$$

where $\underline{\xi}_1=[\underline{\xi};\underline{w}]$. The above augmented description is clearly nonlinear either due to inherent nonlinearities in the system description equations 5.1 and 5.2 and/or due to bilinear product terms consisting of the states in $\underline{x}$ and the parameters in θ. $\underline{z}$ is the measurement vector which, at some instants, could consist of both primary as well as other inferential, secondary measurements. At some other sampling instants, only the secondary measurements could be available. If ns secondary outputs exist, the dimension of $\underline{z}$ would be n+ns or ns depending on the measurement set available at any sampling instant. The secondary measurements relate to those process outputs whose behaviour is correlated with that of the primary states and which can be used with the process model in an inferential estimation scheme to generate estimates of the primary states. i.e. they are process outputs from which the primary states are observable. The secondary measurements are available more rapidly than the primary ones due to availability of robust online sensors. Therefore there exists a multirate sampling scenario where the measurements of the primary states are available only slowly and the secondary measurements are available more rapidly. Such a scenario can be depicted as seen in Figure 5.1. We term the sampling instant at which both the primary and secondary measurements arrive as the major sampling instant and the sampling instant at which only secondary measurements are taken as the minor sampling instant. We also term the time elapsed between two minor sampling instants as the basic sampling time τ.

Many strategies to estimate the primary states from only the secondary measurements have been proposed. These range from the model based Bayesian filtering strategies such as the Kalman filter for linear time invariant systems to the extended Kalman filter(EKF)

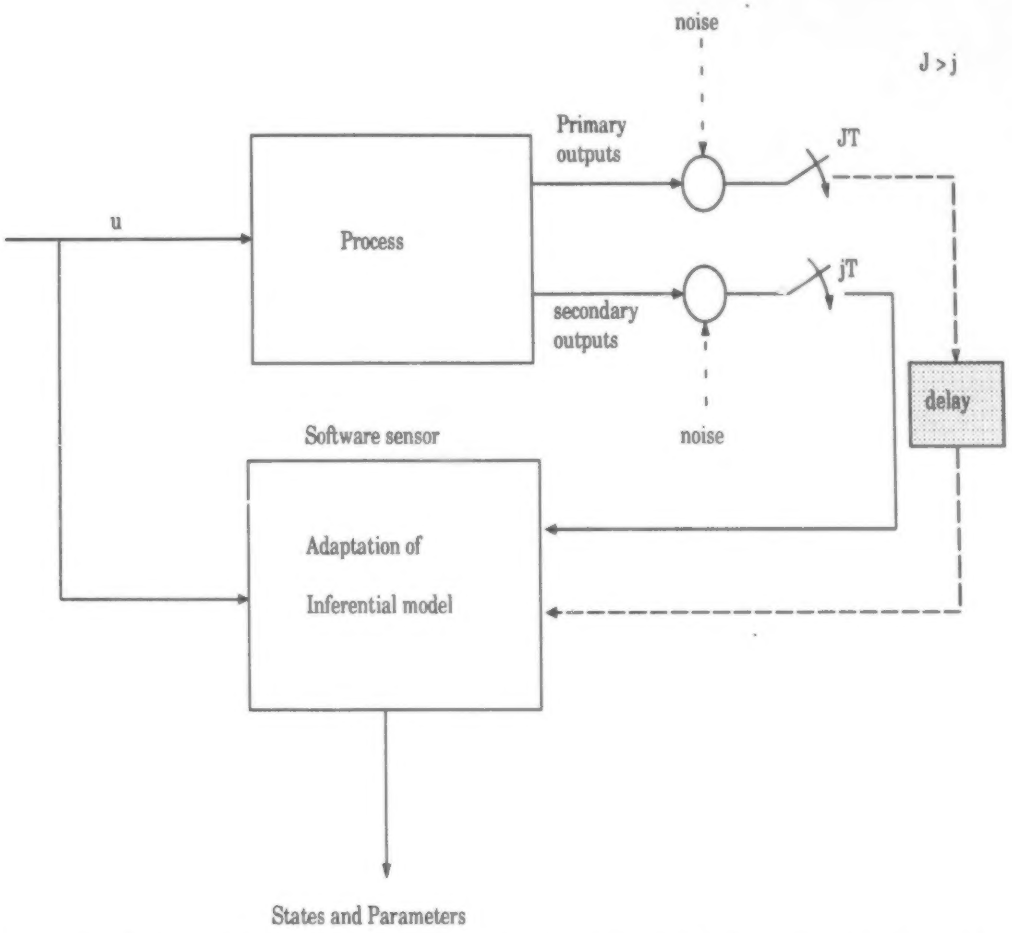

noise
J > j
Primary outputs
JT
u
Process
jT
secondary outputs
delay
Software sensor
noise
Adaptation of
Inferential model
States and Parameters

Figure 5.1 Schematic diagram of multirate sampling for processes commonly encountered in chemical engineering.

for non-linear, time varying systems. In a recursive implementation, the EKF is prone to divergence problems due to repeated linearization of the process model around the filter's latest state estimate. The latter problem causes any initial error/model mismatch to propagate which eventually leads to divergence. When the states in the state vector represent physically measurable variables in the system such as would result from a linearization and discretization of the mass and energy balance equations of a system , it is logical to use these slowly sampled primary measurement as feedback information to the estimator and thus make the estimation more robust. In earlier studies, Gudi *et al.* (1993a,b) used a Kalman filter coupled with a sequential parameter updating strategy in a multirate framework to perform the state and parameter identification. However, it was not possible to tailor this strategy for the case of measurement delay. Furthermore, tuning guidelines for robust performance of the overall algorithm could not be easily expressed. In this chapter, we address the above problems by using a Bayesian approach in an multirate extended Kalman filter framework.

5.3 Multirate Software Sensor

5.3.1 Multirate Iterated Extended Kalman Filter

We first present the equations for the extended form of the linear multirate Kalman filter. Linearizing equation 5.4 around the latest state prediction $\underline{\bar{X}}(i)$ and discretizing using a sampling time τ yields,

$$\delta\underline{X}(i+1) = \phi\ \delta\underline{X}(i) + \Gamma\delta\underline{u}(i) + \xi_1(i) \tag{5.6}$$

where ϕ and Γ are the linearized and discretized system matrices and $\delta(.)$ denotes the perturbation or the deviation operator. At the major sampling instant when the measurements of both, primary as well as the secondary measurements are available, the measurement vector $\underline{z}=\underline{z}_{major}$ is of a larger dimension (n+ns *by* 1). The linearized, discrete measurement equation can be written by linearizing equation 5.5 at the major sampling instant as,

$$\delta \underline{z}(i)_{major} = H_{major}\, \delta \underline{X}(i) + \eta_{major} \tag{5.7}$$

where,

$$H_{major} = \frac{\partial G_{major}}{\partial \underline{X}} |\underline{X} = \bar{\underline{X}}(i) \tag{5.8}$$

is the linearized measurement matrix at the major sampling instant and has dimensions n+ns *by* n+nθ. The measurement noise covariance matrix corresponding to the major sampling instant $R_{z,major} = E(\eta_{major}, \eta_{major}^T)$ would also be a larger diagonal matrix of measurement noise covariances. The Kalman gains can then be evaluated at the major sampling instant as,

$$K_{major} = M\, H_{major}^T\, (H_{major}\, M\, H_{major}^T + R_{z,major})^{-1} \tag{5.9}$$

where M is the *apriori* estimate of the covariance matrix. Note that the Kalman gain matrix (size n+nθ *by* ns+n) is a larger matrix with as many columns as the number of measurements. At the minor sampling instants, only the inferential secondary measurements are available and the measurement vector $\underline{z} = \underline{z}_{minor}$ is of a smaller dimension(size ns *by* 1). The linearized measurement matrix H_{minor} is also a smaller matrix (size ns *by* n+nθ) with as many rows as the number of measurements. The linearized measurement equation can be written as,

$$\delta \underline{z}(i)_{minor} = H_{minor}\, \delta \underline{X}(i) + \eta_{minor} \tag{5.10}$$

where

$$H_{minor} = \frac{\delta G_{minor}}{\delta \underline{X}} |\underline{X} = \bar{\underline{X}}(i) \tag{5.11}$$

The measurement noise covariance matrix $R_{z,minor}$, corresponding to the minor sampling instants would then be a diagonal matrix of measurement noise covariances with an appropriately smaller dimension. The Kalman gains for the minor sampling instant can then be written as

$$K_{minor} = M\, H^{T}_{minor}\, (H_{minor}\, M\, H^{T}_{minor} + R_{z,minor})^{-1} \tag{5.12}$$

Note that for the minor sampling instant, the Kalman gain matrix is a smaller matrix (size n *by* ns) with as many columns as the number of measurements. The overall extended Kalman filter equations in multirate form are therefore the same as the those of the classical extended Kalman filter, but with periodicity in Kalman gains, measurement and process covariance matrices. The two step formulation of the time-varying Kalman filter (Franklin and Powell,1980) can then be used to express the extended Kalman filter in its multirate form as :

a) Major sampling instant :

i)Measurement update:

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{major}\, \underline{\varepsilon}(i) \tag{5.13}$$

$$\underline{\varepsilon}(i) = \underline{z}_{major} - \underline{G}_{major} \tag{5.14}$$

$$P(i) = M(i) - K_{major} H_{major} M(i) \tag{5.15}$$

b) Minor sampling instant :

i)Measurement update:

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{minor}\, \underline{\varepsilon}(i) \tag{5.16}$$

$$\underline{\varepsilon}(i) = \underline{z}_{minor} - \underline{G}_{minor} \tag{5.17}$$

$$P(i) = M(i) - K_{minor}\, H_{minor}\, M(i) \tag{5.18}$$

To perform the measurement update at every sampling instant, predictions of the state X and the covariance M are required. These are obtained using the time update equations by performing an actual integration of the nonlinear augmented system description. Thus,

$$\dot{\underline{X}} = \underline{F}\,(\hat{\underline{X}}, \underline{u}, t) \tag{5.19}$$

$$\dot{M} = F_{\underline{X}}\,P + P\,F_{\underline{X}}^{T} + Q \tag{5.20}$$

where $F_{\underline{X}}$ is the Jacobian of the augmented system description and is given as,

$$A_{(n,n)} = \frac{d\underline{f}}{d\underline{x}} \tag{5.21}$$

$$F_{\underline{X}} = \begin{bmatrix} A & \underline{0} \\ \underline{0} & I_{n\theta,n\theta} \end{bmatrix} \tag{5.22}$$

The measurement update step at any instant could be interpreted as a step towards minimization of the objective function of the estimation/measurement error suitably weighed by the covariance matrices. For linear time invariant systems, the Kalman gain represents the necessary information for this minimization quite accurately and therefore the measurement update step, when performed once, minimizes the relevant objective function. For a nonlinear system however, the extent to which the Kalman gain represents the minimization information is dependent on the reference trajectory around which the system linearization is done. If an *apriori* nominal trajectory is chosen, the information accuracy depends on how close the nominal trajectory is to the true one. If the linearization is done around the latest state estimates, the information accuracy depends on filter performance itself and the objective function may not be necessarily minimized in one step of the measurement update equation. This motivates the use of an iterated version of the extended Kalman Filter equations (Denham and Pines (1966) and Jazwinski (1970)). The iterated extended Kalman Filter (IEKF) has been recommended for strong nonlinearities in the output equation and has been successfully used for fermentation processes by Bellgardt *et al.* (1989), Sargantanis and Karim (1994). The iterated version of the extended Kalman filter equations can be expressed as shown below: Assume that the state prediction $\bar{\underline{X}}(i)$ is available at instant i, then the local recursive scheme can be invoked by initializing $\underline{\gamma}(1)$ to be equal to $\bar{\underline{X}}(i)$ and then recursing over the equation,

$$\bar{\underline{\gamma}}(k+1) = \bar{\underline{X}}(i) + K[\bar{\underline{\gamma}}(k)]\ \{z_i - \underline{G}[\bar{\underline{\gamma}}(k),\underline{u}(i)] - H[\bar{\underline{\gamma}}(k)]\ (\bar{\underline{X}}(i)-\bar{\underline{\gamma}}(k)\ )\}\ ,\ k = 1,\dots,l \tag{5.23}$$

The Kalman gains K and the linearized output matrix H are based on the latest estimate $\underline{\gamma}(k)$. As has been shown by Denham and Pines(1966), significant improvements in the estimates occur in the first few local iterations. Nevertheless, the above equation is iterated until there is no significant improvement in any successive estimates of $\underline{\gamma}$. The above locally iterated EKF based strategy has been used in a multirate framework in this work, for robust estimation. The iterative extended multirate Kalman filter formulation can thus be used to formally incorporate the slowly sampled primary measurement into the overall filtering and estimation scheme. It is important to note that the above multirate formulation does not require a fixed integer ratio of the minor and major sampling instants. In earlier approaches to multirate identification (Lu *et al.* (1990), Guilandoust *et al.* (1988)), using transfer function models, it was required that the major sampling time arrives every fixed integer, say J, sampling intervals of the minor sampling instants. This is too restrictive a requirement for the resulting strategy to be generally applicable at an implementation level. The above multirate Kalman filter formulation is more flexible as it simply uses a different set of equations at the major sampling instants whenever the primary measurements arrive, and otherwise switches back to the inferential equation set at the minor sampling instants.

5.3.2 Measurement delays

Typically, measurement of the primary state could involve sampling followed by elaborate off-line assays in the laboratory thus leading to a delay in the availability of the results of sampling. Such delays can easily be incorporated into the system model equations by addition of delayed states. Consider, for simplicity, the following discrete state space model :

$$\delta\underline{x}(i+1) = \phi\delta\underline{x}(i) + \Gamma\delta u(i) + \underline{w}(i) \tag{5.24}$$

$$\delta\underline{z}(i) = H\delta\underline{x}(i) + \underline{v}(i) \tag{5.25}$$

Assume for simplicity that there is only one sampled state x_1 in the state vector $\underline{x}$. The measurement relationship H is then equal to 1. If the state x_1 is sampled with a measurement delay of t_d time units, the equations to obtain the delayed state x_m in continuous time can be written as,

$$x_m(t) = x_1(t-t_d) \tag{5.26}$$

Assuming for simplicity that the delay time is an integral multiple of the basic sampling time τ, we can write the delay equations for the case of $t_d=2\tau$, in terms of perturbation variables in the discrete domain as,

$$\delta x_2(i+1) = \delta x_1(i) \tag{5.27}$$

$$\delta x_3(i+1) = \delta x_2(i) \tag{5.28}$$

where x_2 and x_3 are the delayed states. The corresponding measurement equation should also then be modified to indicate that the measurement of the primary state arriving at any instant i relates to the delayed state x_3 and not to x_1. Thus, the overall augmented system description can be written as,

$$\delta\underline{X}(i+1) = \phi_1\delta\underline{X}(i) + \Gamma\delta u(i) + \underline{w}(i) \tag{5.29}$$

$$\delta\underline{z}(i) = H_1\delta\underline{X}(i) + \underline{v}(i) \tag{5.30}$$

where,

$$\phi_1 = \begin{bmatrix} \phi & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} \tag{5.31}$$

$$H_1 = [0 \; 0 \; H] \tag{5.1}$$

The augmented system consisting of the basic system states and the delayed states can now be considered as representative of the system behaviour. In general, measurement delays reduce system observability and deteriorate estimator performance. However, as will be shown in the modified observer formulation in section 5.4.4, it is possible to enhance system observability by using past inferential measurements in the measurement equation. Thus, the above equations, along with the modified observer formulation, can be used to effectively address the problems posed by measurement delays in the primary variables.

5.3.3 Comments on System Observability

Implicit in the foregoing development is the basic assumption that the augmented system consisting of the original system states, time varying parameters and delayed states is completely observable from the process outputs. This needs to be verified before the strategy can be used. For non-linear systems, observability properties are relatively difficult to verify (Ray(1981)). It is often recommended that the tools developed for observability analysis of linear systems be used for nonlinear systems by considering a linearized approximation of the latter about some nominal, apriori assumed trajectory. Thus, the observability matrix can be constructed for the linear approximation of the nonlinear system and conditions on the positive definiteness of the information matrix (Jazwinski,1970) or the rank of the observability matrix can be applied to ensure system observability. It must be noted that linear filters constructed for nonlinear systems can still diverge even though the above tests on observability are met. In general, system observability is a "yes" or "no" measure. Ad-hoc measures of observability such as "weakly" or "strongly" observable systems can be obtained by examining the singular values of the observability matrix (Lin(1979),Shah *et al.*(1981)).

In a multirate framework, tests for observability must be carefully carried out because the dimensions of the process output vector varies between the major and minor sampling instant. Ideally, one would like the system to be completely observable from the secondary process outputs alone. However, the availability of the slowly sampled primary measurement at the major sampling instant can serve to make the estimator performance more robust in face of uncertain initial conditions and parameters by providing additional feedback information to the estimator rather than relying on feedback from estimates generated from the secondary outputs alone. The availability of the primary measurement also serves to update a larger set of system parameters, if necessary, and may thus help to make the system strongly observable.

5.4 Case Study

Analysis of the proposed software sensor has been carried out on simulation and experimental data from fermentation systems. Such systems exhibit significant time varying behaviour due to changing conditions in the growth medium throughout the fermentation. For example, the specific growth rate and maintenance coefficient vary with time. In fermentations of recombinant organisms, the growth rate could change due to induction of the plasmids. Fermentations exhibiting diauxic growth phenomenon can also exhibit time varying specific growth rates due to a change in the type of nutrients being assimilated. The system considered in this case study is a fed batch antibiotic producing fermentation known to exhibit significant maintenance activity and endogenous metabolism during the secondary product (antibiotic) expression phase.

For numerical simulation , the system was modelled using dynamic balances along with empirical growth models for cell growth and product formation (Bajpai and Reuss (1980), Tsobanakis *et al.* (1991)) and dynamic balances for the gas phase (Cardello and San(1988)). The detailed equations are presented in Appendix A. The system was simulated using a stiff equation solver LSODES (Hindmarsh (1983)) that is available from the Lawrence Livermore Laboratories. The profiles of various states thus generated by simulation were then sampled according to a multirate sampling scheme chosen for the study.

5.4.1 Estimator Equations

Inferential estimation of the biomass, net specific growth rate μ and the maintenance coefficient m_c was proposed to be carried out from (i) frequent and regular measurements of the carbon dioxide evolution rate (CER) available at the basic sampling rate and (ii) infrequent, delayed measurements of the biomass available at a slower sampling rate which is assumed to be some integral multiple of the basic sampling rate.

The balance equation for the cumulative amount of biomass x_1(=XV) can be written by performing a simple transformation of the dynamic balance equation for the biomass (equating $r_X = \mu X$ in equation A.1 of the Appendix) as,

$$\frac{dx_1}{dt} = \mu\, x_1 \tag{5.2}$$

Commonly, structural models, such as the Monod model, that relate the growth rate to biomass and substrate concentrations are used to explain the variation in μ. In the above equation however, μ is assumed to be a time varying parameter and no growth model is assumed to describe it. A measurement delay t_d = 2 basic sampling instants was assumed for the biomass. In continuous time, the following equation simulates the delay.

$$x_m(t) = x_1(t-t_d) \tag{5.3}$$

However, two delayed states need to be introduced as discussed in section 3.2, to simulate the delays in the discrete domain. The discrete, output equation can be expressed in terms of the CER (mmol/h) by assuming the gas phase dynamics to be significantly faster than the rates of growth or substrate consumption. Thus from equation A.7 in Appendix A,

$$CER(i) = [k_1\mu(i) + m_c(i)]\ x_1(i) \tag{5.4}$$

where k_1 is the yield coefficient.

The measurement equation at the major sampling instant, when we have measurements of the cumulative biomass and CER, can be written as,

$$\underline{z}_{major} = \begin{bmatrix} x_3(i) \\ CER(i) \end{bmatrix} + \underline{v}_{major} = \begin{bmatrix} x_3(i) \\ (k_1\mu(i)+m_c(i))x_1(i) \end{bmatrix} + \underline{v}_{major} \tag{5.5}$$

At the minor sampling instant, measurement of only CER is available and the measurement equation can be written as,

$$\underline{z}_{minor} = CER(i) + \underline{v}_{minor} = \left[k_1\mu(i) + m_c(i)\right] x_1(i) + \underline{v}_{minor} \tag{5.6}$$

The specific growth rate μ and the maintenance coefficient m_c are time varying and need to be estimated in addition to the states. They are modelled as discussed earlier as,

$$\dot{\mu} = w_2 \tag{5.7}$$

$$\dot{m}_c = w_3 \tag{5.8}$$

where w_2 and w_3 are zero-mean noise processes with unspecified probability densities.

5.4.2 Observability analysis

For time varying, nonlinear systems, observability conditions can be checked by considering linearized approximations. Structural unobservabilities, if any, would render the system unobservable at all sampling instants. These can be easily detected if the relevant observability matrices are constructed symbolically and then checked for rank deficiencies. The results of such a symbolic observability analysis are independent of the values that the parameters can take and are thus generic. For the above nonlinear system of equations, we perform a structural observability analysis using Maple (1981). We consider the observability of the system in a locally linearized sense under the following scenarios:

(i) System with no measurement delays

The system equations that need to be considered here are (5.33), (5.38) and (5.39)

The state transition matrix after linearization and discretization of the above system equation can be written as,

$$\phi = \begin{bmatrix} 1+\tau\mu(i) & \tau x_1(i) & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{5.9}$$

At the major sampling instants, measurements of biomass and CER are available and the linearized measurement equation is,

$$h_{major} = \begin{bmatrix} 1 & 0 & 0 \\ k_1\mu(i)+m_c(i) & k_1x_1(i) & x_1(i) \end{bmatrix} \tag{5.10}$$

The corresponding structural observability matrix, $O_{major}=[h_{major};\ h_{major}\phi;\ h_{major}\phi^2]^T$ can be written as,

$$O_{major} = \begin{bmatrix} 1 & 0 & 0 \\ k_1\mu(i)+m_c(i) & k_1x_1(i) & x_1(i) \\ (1+\tau\mu(i)) & \tau x_1(i) & 0 \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i)) & (k_1\mu(i)\ +\ m_c(i))\tau x_1(i)+k_1x_1(i) & x_1(i) \\ (1+\tau\mu(i))^2 & \tau x_1(i)(2+\tau\mu(i)) & 0 \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i))^2 & (k_1\mu(i)+m_c(i)(2+\tau\mu(i))\tau x_1(i)\ +\ k_1x_1(i) & x_1(i) \end{bmatrix} \tag{5.11}$$

Using Maple, this matrix can be shown to be of full rank and therefore the augmented system of the states and the parameters is completely observable from the process outputs at the major sampling instant.

At the minor sampling instants, since only measurements of the CER are available, the linearized measurement equation can be written as,

$$h_{minor}=[(k_1\mu(i)+m_c(i)) \quad k_1x_1(i) \quad x_1(i)] \qquad (5.12)$$

The observability matrix for this case can be verified to be,

$$O_{minor} = \begin{bmatrix} k_1\mu(i)+m_c(i) & k_1x_1(i) & x_1(i) \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i)) & (k_1\mu(i)+m_c(i))\tau x_1(i)+k_1x_1(i) & x_1(i) \\ (k_1\mu(i)+m_c(i))(1+\tau\mu(i))^2 & (k_1\mu(i)+m_c(i))(2+\tau\mu(i))\tau x_1(i)+k_1x_1(i) & x_1(i) \end{bmatrix} \qquad (5.13)$$

The above matrix can be shown to be rank deficient. It has a rank of 2 i.e. a rank defect of 1. The system is thus structurally unobservable, so that the overall system of states and parameters is only partially observable from the secondary measurements. From intuition and from systems theory (such as the directed mode expansion of the output), it is easy to verify that at minor sampling instants, only the state x_1 and the specific growth rate μ are observable from the CER. Thus if the maintenance coefficient m_c is constant, state estimation can still be done.

(ii) System with measurement delay

The system equations that need to be considered for this case are (33),(34),(38) and (39). The state transition matrix after linearization and discretization can be written as,

$$\phi = \begin{bmatrix} 1+\tau\mu(i) & 0 & 0 & \tau x_1(i) & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \qquad (5.14)$$

Major sampling instant

The measurement of the biomass that is available at this sampling instant corresponds to the delayed state x_3. Thus the measurement equation can be written as,

$$z_{major,delay} = \begin{bmatrix} x_3(i) \\ (k_1\mu(i)+m_c(i))x_1(i) \end{bmatrix} + \underline{v}_{major,delay} . \tag{5.15}$$

The linearized measurement equation thus is,

$$h_{major,delay} = \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ k_1\mu(i)+m_c(i) & 0 & 0 & k_1x_1(i) & x_1(i) \end{bmatrix} \tag{5.16}$$

Using Maple, the observability matrix (omitted here for brevity) can be constructed and verified to be of full rank. Thus even for the case of measurement delay, the system is fully observable at the major sampling instant.

Minor Sampling instant

The measurement equation remains the same as the case of no measurement delay since the delayed biomass measurements are not available at this sampling instant at all. The state vector, however, does include the delayed states and the linearized measurement equation can be written as,

$$h_{minor,delay} = \begin{bmatrix} k_1\mu(i)+m_c(i) & 0 & 0 & k_1x_1(i) & x_1(i) \end{bmatrix} \tag{5.17}$$

Using Maple, the observability matrix for this case can be found to be rank deficient with a rank of 2.

Compared to the case of minor sampling instant with no measurement delay, we see that that there is an increase in the rank defect. From intuition and from systems theory, it is easy to see that this increase in rank defect of 2 is due to the addition of the delayed states at the minor sampling instant. These delayed states are not correlated with the system output (CER) and are thus not observable at the minor sampling instants. Thus at the minor sampling instants, when there is delay, the maintenance coefficient and the delayed states are not observable.

5.4.4 Modified Observer

From the preceding observability analysis, it is then possible to conclude that

(i) the system with measurement delay is fully observable at the major sampling instants,

(ii) At the minor sampling instants, the system is unobservable.

One obvious implementation of the estimator for the case of undelayed measurement is to update the maintenance coefficient at the major sampling instant and to assume that the major sampling instants arrive frequently enough to track the changing maintenance coefficient. For the case with measurement delay, however, one can then extend the same ideas, provided of course that it is possible to make the delayed states observable in some way. This motivates the use of a modified observer presented below.

The basis for the modified observer formulation lies in the fact that past inferential measurements must be correlated with the delayed states. Since, we know that the rank defect at the minor sampling instant increases by the number of added delayed states, by including past measurements in the observation equation, we can attempt to decrease the rank defect by the same number. The motivation for doing so can also be drawn in analogy with the philosophy of adding delayed states. The latter are only hypothetical states added to simulate delay in the state space description i.e. for mathematical tractability. Thus, to observe them we can add as many delayed measurements i.e. past measurements in the measurement equation. For the above minor sampling instants, the measurement equation can then be written as,

$$\underline{z}_{minor,modified}=\begin{bmatrix} CER(i) \\ CER(i-1) \\ CER(i-2) \end{bmatrix} + \underline{v}_{minor,modified} \tag{5.18}$$

If we now make the additional assumption that μ and m_c remain constant during the measurement time delay (a valid assumption considering the dynamics of the bioprocess), we can write

$$\underline{z}_{minor,modified} = \begin{bmatrix} (k_1\mu(i)+m_c(i))x_1(i) \\ (k_1\mu(i)+m_c(i))x_2(i) \\ (k_1\mu(i)+m_c(i))x_3(i) \end{bmatrix} + \underline{v}_{minor,modified} \tag{5.19}$$

The linearized measurement matrix can be written as

$$h_{minor,modified} = \begin{bmatrix} k_1\mu(i)+m_c(i) & 0 & 0 & k_1x_1(i) & x_1(i) \\ 0 & k_1\mu(i)+m_c(i) & 0 & k_1x_2(i) & x_2(i) \\ 0 & 0 & k_1\mu(i)+m_c(i) & k_1x_3(i) & x_3(i) \end{bmatrix} \tag{5.20}$$

Using Maple, the observability matrix for the linearized system with the modified measurement equation can be verified to be of full rank. Comparing the results obtained at the minor sampling instants in this section with the modified measurement equation with those obtained in section 5.4.3, it is possible to conclude that by inclusion of the past measurements and by exploiting the system structure it has been possible to decrease the rank defect to zero and make the system completely observable.

As mentioned earlier, ad-hoc measures of observability such as "weakly" or "strongly" observable systems can be obtained by examining the singular values of the observability matrix. Thus, a system could be termed as "strongly" observable if it had a well conditioned observability matrix. In the foregoing analysis, although the observability matrix was of full rank at the major sampling instants indicating that the system was fully observable at these instants, past measurements were still used in a modified measurement equation to improve the condition number of the resulting observability matrix and thus make the system more strongly observable at the major sampling instants.

Thus at the major sampling instants, the measurement equation is written as,

$$\underline{z}_{major,modified} = \begin{bmatrix} x_3(i) \\ (k_1\mu(i)+m_c(i))x_1(i) \\ (k_1\mu(i)+m_c(i))x_2(i) \\ (k_1\mu(i)+m_c(i))x_3(i) \end{bmatrix} + \underline{v}_{major,modified} \tag{5.21}$$

The linearized measurement equation can be written as

$$h_{major,modified} = \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ k_1\mu(i)+m_ci & 0 & 0 & k_1x_1(i) & x_1(i) \\ 0 & k_1\mu(i)+m_c(i) & 0 & k_1x_2(i) & x_2(i) \\ 0 & 0 & k_1\mu(i)+m_c(i) & k_1x_3(i) & x_3(i) \end{bmatrix} \tag{5.22}$$

It was verified using symbolic computation that the observability matrix, when the modified observer equations were used at the major sampling instants, was also of full rank.

Thus the system which initially had structural unobservabilites at the minor sampling instants has been made fully observable at all sampling instants by including past measurements in the system description.

- *Remark* 1: The results of the above structural observability analysis can be said to be *generic*, and applicable to all systems having a similar structure. However, their *validity* as applied to nonlinear systems, is restricted to the locally linearized system description.
- *Remark* 2: Such delayed and/or infrequent sampling of the primary process variables, in a multirate scenario as presented above, is present in many chemical processes which involve sampling and elaborate off-line analysis or assay procedures. The structural unobservabilities seen above are also typical of many chemical process systems. For example, in an exothermic batch reactor, the temperature of the reactor contents is influenced by the reaction as well as the heat transfer rates. Commonly, reactor temperature measurements are used to infer typical reactor

states (reactant compositions) and parameters (reaction rates) (Bonvin *et.al* (1989),de Valliere and Bonvin (1989)). If the heat transfer rates change due to a change in the overall heat transfer coefficient, the state and parameter estimates, generated from temperature measurements alone, could be biased. It would then be necessary to update the heat transfer coefficient in exactly the same way as the maintenance coefficient discussed above, to enhance the quality of the state and parameter estimates.

5.5 Estimation Results

Simulation study

The system of equations presented in Appendix A was solved for a time interval of 120 hours for a linearly changing value of the maintenance coefficient m_c between 1.1 to 1.6 mmol CO_2/h-l. A constant nutrient feed rate of 5.6 ml/h was assumed. The profiles for biomass concentration, CO_2 content in the exit gas, the specific growth rate and the maintenance coefficient thus generated are shown in Figure 5.2.

A multirate sampling scenario was chosen as follows. Measurement of the biomass was assumed to arrive every 3 hours from the simulations. Measurements of the CO_2 content in the exit gas and the broth volume were assumed to arrive every 6 minutes. In terms of the sampling instants, the major sampling instant arrived every 3 hours or after every 30 minor sampling instants. A delay of 2 minor sampling instants was assumed for the primary measurement. White Gaussian noise with zero mean was added to the measurements to simulate noisy measurements with a relatively smaller signal to noise ratio. The objective of the estimator was thus to estimate, from the measurements arriving at multiple rates of sampling described above, the cumulative biomass, the specific growth rate and the maintenance coefficient.

Base case Estimation : Figure 5.3. shows the behaviour of the estimator for the base case estimation. It can be seen that state and parameter estimation is excellent. The parameters are tracked quite well but the estimates are noisy due to the high sensitivity of the filter to the measurement noise added. For the above run, the process noise covariance matrix

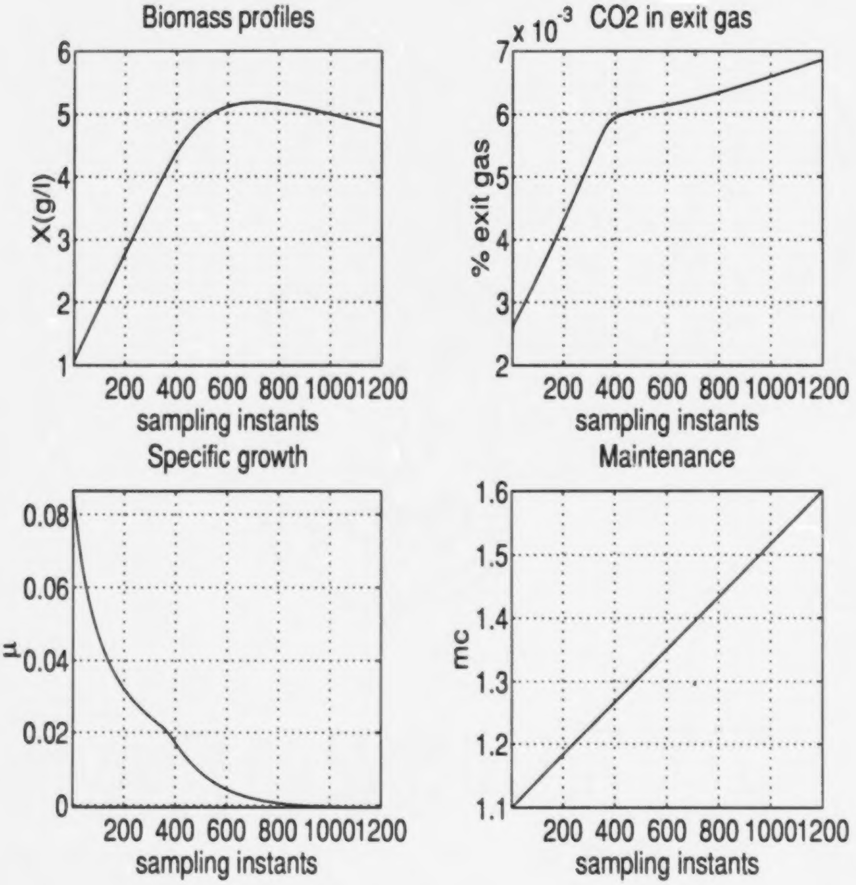


Figure 5.2 Profiles of process variables and parameters from simulation of a fed batch antibiotic fermentation

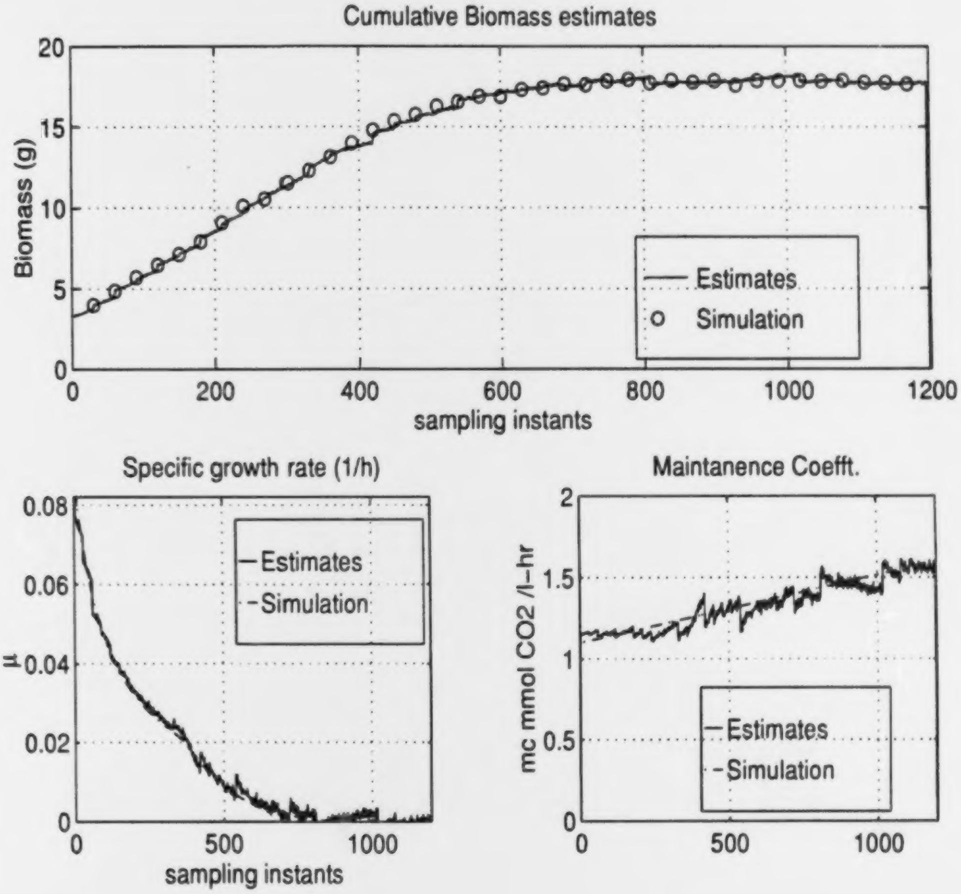


Figure 5.3 Performance of multirate EKF based estimation strategy using simulated data (Base case).

TABLE 5.1

Process noise covariance matrices for the simulation runs.

Validation case	Process noise covariance matrix	Figure number in the text of the chapter.
Base case estimation	1000 times diagonal(1,1,1,50,50).	Figure 5.3
Effect of major sampling interval	1000 times diagonal(1,1,1,50,50).	Figure 5.4
Effect of measurement delay	1000 times diagonal(1,1,1,50,50).	Figure 5.5
Effect of modified parameter covariance	1000 times diagonal(1,1,1,10,10).	Figure 5.6

Q was assumed to be a diagonal matrix with entries corresponding to the "process noise" levels of each of the states. The measurement noise covariance matrix was also assumed to be 0.1 times an appropriately sized identity (diagonal) matrix with entries corresponding to the covariance of measurement noise. The values of the process noise covariance matrices that were used in the estimation run are mentioned in Table 5.1.

The cumulative biomass profiles show corrections on arrival of a biomass measurement at the major sampling instants. This is through the appropriate specification of the process and measurement noise covariances such that greater confidence is placed on the estimates of the biomass generated from primary measurements that are available at any sampling instant as opposed to the estimates generated inferentially from the CER.

Effect of Major sampling frequency : Figure 5.4 shows the performance of the estimator when the major sampling instant occurs every 60 minor sampling instants. It can be seen that, due to slower availability of the primary measurement, the tracking of the true parameters is sluggish. This can also be judged from the drastic corrections made by the estimator at the major sampling instant to generate the biomass estimates. These drastic corrections indicate that, due to slower tracking of the parameters, the estimates of the biomass generated inferentially from the CER at the minor sampling instants do not track the true values correctly.

Effect of Measurement delay : Figure 5.5 depicts the performance of the estimator when there is a measurement delay of 30 sampling instants. Comparing this case with the base case (Figure 5.3), it is easy to conclude that the estimator can robustly accommodate measurement delays. The estimator run in Figure 5.5 corresponds to a sampling regime where a primary sampling is made when the results of the previous measurement is obtained. Due to large delays in measurement availability, the tracking of the parameters is quite sluggish.

Effect of Parameter covariance: Figure 5.6 depicts the estimator performance when the parameter covariance matrix assumes values 10,000 times the appropriate identity matrix. For the extended Kalman filter formulation used in this work, this parameter covariance

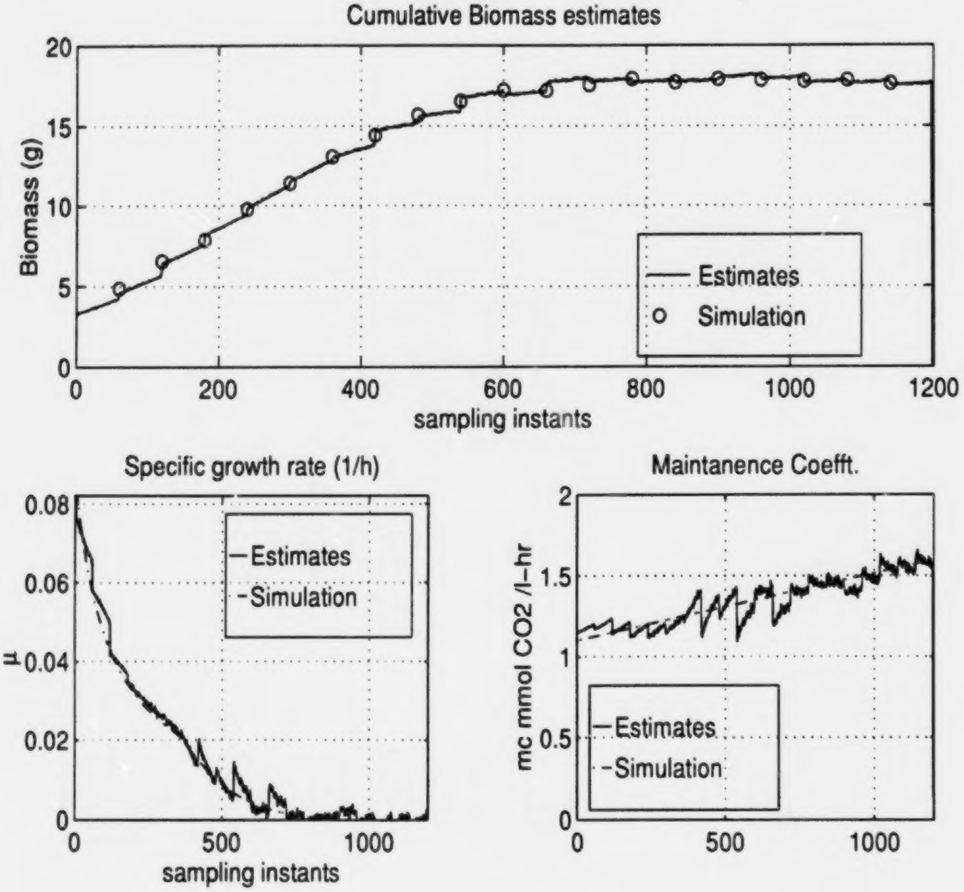


Figure 5.4 Effect of an increase in the major sampling interval on the estimator performance (Major sampling instant every 60 minor sampling instants).

matrix is a subset of the overall process noise covariance matrix. The measurement noise covariance matrix was assumed to be 0.1 times the appropriate identity matrix for both the major and minor sampling instants.

Figure 5.6 shows that biased parameters are obtained for a small value of the parameter covariance. However, the tracking of the primary state is quite good. Furthermore, because the tracking of the parameters is sluggish, these trajectories are quite smooth and are not sensitive to the measurement noise at all. As the parameter covariance is increased, the parameter estimates become less biased and converge to the true values. However when the parameter covariance is increased, the filter becomes more and more sensitive to the measurement noise and the parameter estimates are noisy. This can be seen by comparing parameter trajectories in Figure 5.3 and Figure 5.6.

5.6 Conclusions

- An algorithm to formally accommodate the multiple rates of sampling that typically exist in chemical processes has been proposed. It has been shown to have a sound theoretical basis by formulating it as an extended version of the multirate Kalman filter.
- Measurement delays associated with the primary measurement are formally incorporated into the system description. The subsequent decrease in system observability due to incorporation of the delays, if any, is addressed by using past measurements in the measurement vector to annul the rank deficiency of the observability matrix.
- The algorithm has also been shown to not have restrictive assumptions on the frequency of primary and secondary measurements as in the transfer function based approaches proposed earlier in the literature. These features allow the estimator to be easily implementable in a typical real time sampling scenario involving measurement delays and irregularities in measurement availability.
- The algorithm has been validated on a case study involving fermentation systems. Validation using both simulation and experimental data from an antibiotic fermentation has been successfully carried out (Gudi *et al.*(1994)). The practicality of the proposed algorithm has thus been confirmed by an experimental application.

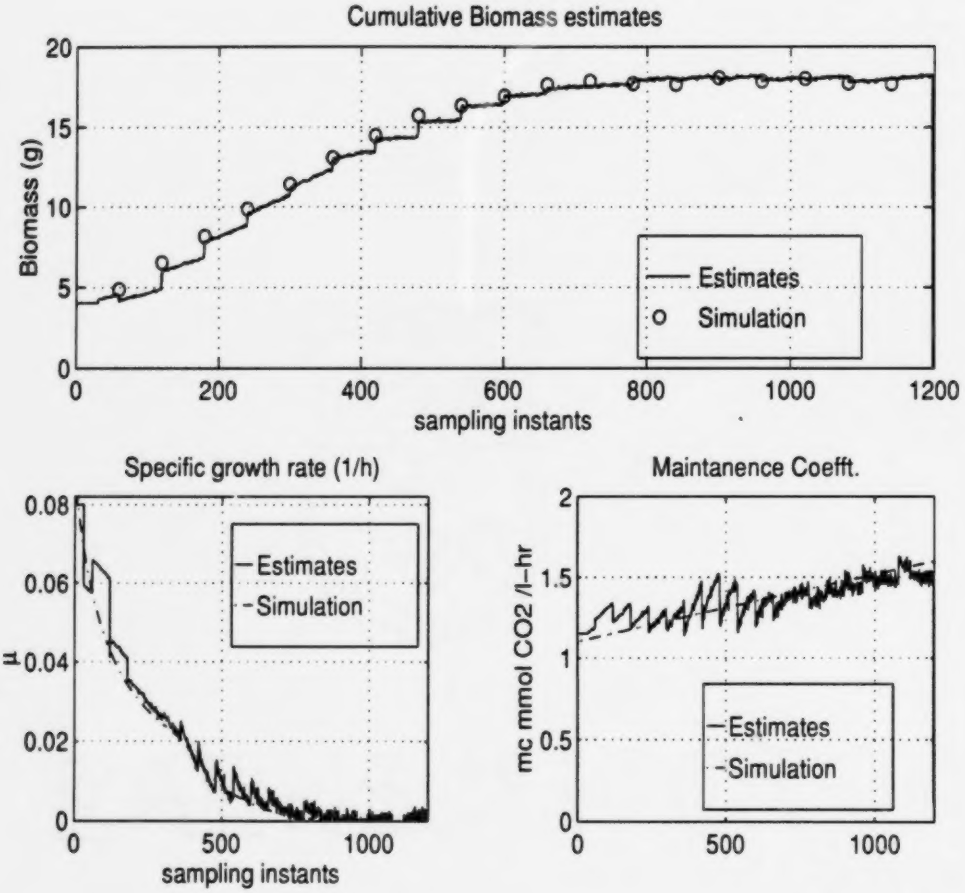


Figure 5.5 Effect of a measurement delay of 30 sampling instants on parameter estimation with simulated data.

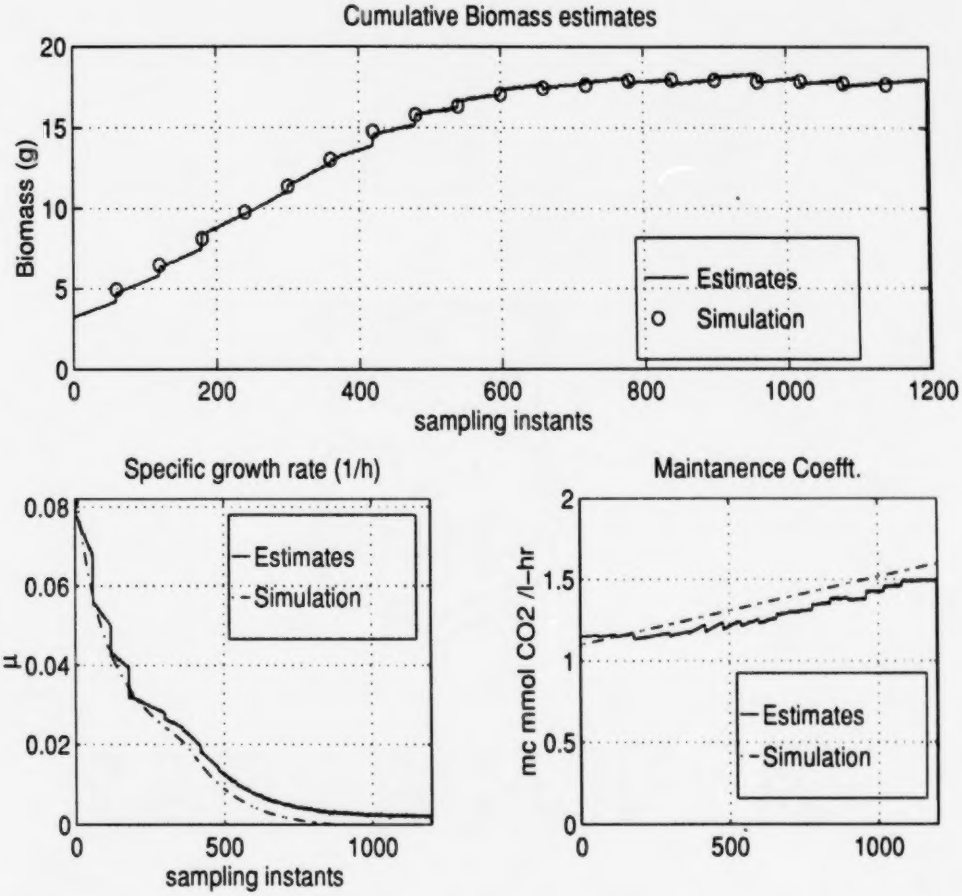


Figure 5.6 Estimator performance on simulated data with modified parameter covariance.

5.7 References

Bajpai, R.K and Reuss, M., " A mechanistic model for Penicillin Production", J. Chemical Technology and Biotechnology,**30**,p 332,(1980)

Bellgardt, K., Kuhlmann, W., Meyer, H., Schugerl, K. and Thoma, M.,"Application of an Extended Kalman filter for state estimation of a yeast fermentation", IEE proceedings, **133**(5), p 226-234, (1986).

Bonvin, D., de Valliere, P. and Rippin, D.W.T., "Application of estimation techniques to batch reactors -I Modelling and thermal effects", Computers and Chemical Engineering, **13**(1), p 1-9, (1989).

Brosilow, C. and Tong, M.,"The structure and dynamics of Inferential control systems", AIChE Journal, **24**(3), p 492,(1978)

Bastin, G. and Dochain, D.,"Online estimation and adaptive control of Bioreactors", Elsevier Science Publishing Co. Inc., New York, USA (1990).

Cardello, R.J and San, K.Y., "The design of controllers for batch bioreactors", Biotechnology and Bioengineering, **32,** p519, (1988).

Chattaway, T. and Stephanopoulos, G., "Adaptive estimation of bioreactors: monitoring lasmid instability", Chemical Engineering Science, **44**(1), p41, (1989)

DeCarlo, R.A., Linear Systems, Prentice-Hall, Englewood Cliffs, New Jersey, USA, (1989)

Denham, W.F and Pines, S., "Sequential estimation when measurement function nonlinearity is comparable to measurement error" AIAA Journal, **4**, p1071, (1966).

de Valliere, P., and Bonvin, P., "Application of estimation techniques to batch reactors - II Experimental studies in state and parameter estimation", Computers and Chemical Engineering, **13**(1), p 11-20, (1989).

Esener, A.A., Roels, J.A. and Kossen, N.W.F., "Fed-batch Culture : Modelling and Applications in the study of Microbial Energetics", Biotechnology and Bioengineering, **22**, p 1851, (1981)

Franklin, G.F., Powell, J.D. and Workman, M.L., "Digital control of dynamic systems", Addison-Wesley Publishing Co. Inc., Reading, Massachussets, USA(1990).

Glasson, D.P., " Research in Multirate estimation and control", Analytic Sciences Corporation, Six Jacob Way,Reading, Massachussets (1980).

Glasson, D.P., "Development and Applications of Multirate Digital Control ", IEEE Control Systems Magazine, **3**(4), p 2,(1983).

Gudi, R.D., Shah, S.L., " The Role of Adaptive Kalman filter as a software sensor and its application to a bioreactor", Proceedings of the 12th IFAC World Congress, **8**, p221, Sydney, Australia,(1993a) .

Gudi, R.D., Gray, M.R. and Shah, S.L., "Multirate estimation and monitoring of Process Variables in a bioreactor", Proceedings of 2nd IEEE Conference on Control Applications, Vancouver, p 487-492, (1993b).

Gudi, R.D., Shah, S.L. and Gray, M.R., " Multirate State and Parameter Estimation in an Antibiotic Fermentation with Delayed Measurements", Biotechnology and Bioengineering, **44**, p1271-1278, (1994).

Guilandoust, M.T., Morris, A.J. and Tham, M.T., " Adaptive Inferential Control", IEE Proceedings, Pt D., **134** (3), p171-179, (1987).

Guilandoust, M.T., Morris, A.J. and Tham, M.T., "An adaptive estimation algorithm for Inferential Control", I.E.C Research, **27**, p1658, (1988).

Halme, A., Kuismin, R. and Koteniemi, M.," A method to consider delayed laboratory analysis in state and parameter estimation of Bioreactors", 1st IFAC symposium on Modelling and Control of Bioprocesses, Pergammon Press,(1985).

Hindmarsh, A.C., " Odepack : A systematized collection of ODE solvers", *In* "Scientific Computing", (Ed) Stepleman, R.S., p55-64, North-Holland Publishing Co., Amsterdam, (1983).

Jazwinski, A.H., "Stochastic Processes and filtering theory ", Academic Press, New York, USA (1970).

Joseph, B. and Brosilow, C.,"Inferential control of chemical processes - parts I,II and III", AIChE Journal, **24**(3), p485,(1978).

Lawrence Livermore National Laboratories, Livermore, CA 94550.

Ljung, L. and Soderstrom, T., "Theory and Practice of recursive Identification", MIT Press, Cambridge, USA (1983).

Lin, C.T., "Structural Controllability", IEEE Transaction on Automatic Control, **19**, p201, (1974).

Lu, W. and Fisher, D.G.," Multirate Constrained Adaptive Control", International Journal of Control, **51**, p1439, (1990).

Maple, Waterloo Maple Software, 450 Phillip St., Waterloo, Ontario, Canada N2L 5J2, (1981).

Maybeck, P.S., "Stochastic Models, Estimation and Control", Vol. 2, Academic Press Inc., New York, USA (1982).

Moore, J.B. and Ledwich, G.," Multivariable adaptive parameter and state estimators with convergence analysis", J. Aust. Math. Soc. Ser. B.,**21**, p176-197, (1979).

Morari, M. and Stephanopoulos, G., "Minimizing unobservability in Inferential Control schemes", International Journal of Control, **31**(2), p367, (1980).

Mou, D.G. and Cooney, C.L., " Growth Monitoring and Control through Computer-aided on-line mass balancing in a fed-batch Penicillin fermentation", Biotechnology and Bioengineering., **25**, p225, (1983).

Park, S. and Ramirez, W.F., "Optimal regulatory control of bioreactor nutrient concentration incorporating system identification", Chemical Engineering Science, **45**(12), p3467, (1990).

Pirt, S.J. and Righelato, R.C.," Effect of growth on the synthesis of penicillin by *Penicillium chrysogenum* in batch and chemostat cultures", Applied Microbiology, **15**, p1289, (1967).

Ramirez, W.F., "Optimal state and parameter Identification : an application to batch fermentation", Chemical Engineering Science, **42**(11), p2749, (1987).

Ray, W.H., "Advanced Process Control", McGraw-Hill Book Co., New York, USA, (1981).

Reda, K.D. and Omstead, D.R., " Automatic fermentor sampling and stream analysis", In " Computer Control of Fermentation Processes", Ed. D.R. Omstead, CRC Press, Boca Raton, Florida, USA (1990).

Sargantanis, J. and Karim, M.N., "Multivariable iterative extended Kalman filter based adaptive control: Case study - Solid Substrate Fermentation", (To appear in Industrial and Engineering Chemistry, Research (1994)).

Shah, S.L., Fisher, D.G., and Seborg, D.E., " Design and experimental evaluation of controllers for Process Undisturbability", AIChE Journal, **27**(1), 131, (1981).

Stephanopoulos, G., and San, K.Y.," Studies on on-line bioreactor identification", Biotechnology and Bioengineering, **26**, p1176, (1984).

Stone, K.M., Thornhill, N.F., Roche, F.W and Fish, N.M," A method of using off-line measurements in an on-line estimator of biomass concentration for a penicillin fermentation and its effects on the quality of the estimates", 2nd IFAC symposium on Modelling and Control of Biotechnological processes and 5th International Conference on control applications to Fermentation Technology, Keystone, Colorado,(1992).

Tham, M.T., Montague, C.A., Morris, A.J. and Lant, P.A., " Soft sensors for process estimation and inferential control", Journal of Process Control, **1**, p3,(1991).

Tsobanakis, P., Lee, S.H., Phillips, J.A., and Georgakis, C., "Issues in the control and optimization of fed-batch bioreactors by tendency models", AIChE National meeting, Nov 14-15,1990.

5.8 Nomenclature

Roman

f	nonlinear process model for a general plant
g	nonlinear measurement function for a general plant
u	manipulative variables of the process
t	time variable

t_d	time elapsed due to measurement delay
w	white noise vector
x	vector of system states
F	nonlinear function for the augmented system description
G	nonlinear measurement function for the augmented system
H	linearized measurement matrix
K	Kalman gain at any sampling instant
M	a priori estimate of the covariance of estimation errors
P	a posteriori estimate of the covariance of estimation errors
Q	covariance of process noise
X	superstate in the augmented system description
Greek	
Γ	control matrix in the discrete state space model
γ	dummy variable used for iterating in the IEKF formulation
$\delta(.)$	deviation or perturbation operator
ε	vector of innovations at any sampling instant i
η	vector of measurement noise
θ	vector of time varying system parameters
μ	specific growth rate of the microorganisms
ν	measurement noise vector
ξ	vector of process noise
τ	sampling time
ϕ	discrete state transition matrix
Superscripts	
^	state estimate via the measurement update equation
-	state prediction via the time update equation
Subscripts	
major	pertaining to the major sampling instant

minor	pertaining to the minor sampling instant
z	pertaining to the measurement vector

Chapter 6

Multirate Adaptive Estimation in an Antibiotic Fermentation with delayed measurements[1]

This chapter discusses issues related to estimation and monitoring of fermentation processes that exhibit endogenous metabolism and time varying maintenance activity. Such culture related activities hamper the use of traditional, software sensor based algorithms such as the extended Kalman filter(EKF). In the approach presented here, the individual effects of the endogenous decay and the true maintenance processes have been lumped to represent a modified maintenance coefficient, m_c. Model equations that relate the measurable process outputs such as the carbon dioxide evolution rate (CER) and biomass to the observable process parameters such as net specific growth rate and the modified maintenance coefficient are proposed. These model equations are used in an estimator that can formally accommodate delayed, infrequent measurements of the culture states such as the biomass as well as frequent, culture related secondary measurements (such as the CER). The resulting multirate software sensor based estimation strategy is used to monitor biomass profiles as well as profiles of critical fermentation parameters such as the specific growth for a fed batch fermentation of *Streptomyces clavuligerus*.

---

[1] A version of this chapter has been published as: R.D. Gudi, S.L. Shah and M.R.Gray, "Multirate state and parameter estimation in an antibiotic fermentation with delayed measurements", *Biotechnology and Bioengineering*, **44**, pp. 1271-1278, (1994).

6.1 Introduction

Monitoring of process variables on a regular basis in a bioreactor continues to be a formidable task due to the lack of adequate sensors that can provide measurements of the key culture states on an online and regular basis. In addition to being able to perform tasks such as fault diagnosis, such monitoring may also be necessary to formulate online control policies designed for optimal operation of the fermentation run. In the latter case, it may also be necessary to generate estimates of key parameters,such as the specific growth rate, to design an effective control policy for the fermentor.

Inferential estimation strategies have largely been used to generate estimates of the key variables such as the biomass and substrate concentrations and key parameters such as the specific growth rate(Stephanopoulos and San (1984). In such strategies, the key variables and parameters are estimated from other correlated measurements of secondary variables such as the carbon dioxide evolution rate (CER),assuming certain systems related conditions such as *observability* are met. The concept of observability is central to the design of state estimators. Loosely speaking, a system is said to be observable, if all its states can be estimated from a knowledge of the model and some measurements of a linear combinations of a small subset of the states. Even when such observability conditions are not met, Bastin and Dochain (1990) have shown that it is possible to design asymptotic observers whose convergence characteristics and performance would then depend on experimental conditions.

Inferential estimation strategies mentioned above have their basis in Bayesian methods such as the Extended Kalman filter (EKF) algorithm to simultaneously estimate the key states and parameters. Pioneering efforts (Stephanopoulos and San (1984), Bastin and Dochain (1990)) in the use of such strategies for estimation have shown them to be workable on biochemical processes. The convergence properties of these estimators are however known to be very sensitive to modeling errors and errors in specifying initial conditions. To address these problems, such algorithms have also been used in an iterated framework (Bellgardt *et al.* (1986)).

The concept of maintenance energy of a growing population was first introduced by Herbert (1958) and Pirt(1965) to explain the observed variation in the yield coefficient Y_{sx} that appears in the Monod model. Roels (1983) has also related the maintenance coefficient to endogenous metabolism which results in a decrease in the biomass. From an inferential estimation viewpoint, a changing maintenance coefficient alters the correlation between the biomass growth and the CER and therefore ignoring the time varying nature of the maintenance coefficient can affect the performance of the estimator. This was implicitly shown in the work of Mou and Cooney (1983) who, for an antibiotic fermentation, used a different inferential correlation during the secondary production phase when the maintenance coefficient can be expected to change due to significant endogenous metabolic activity.

In a typical bioprocess environment, it is common to measure the key variables, such as the biomass concentration, irregularly on an off-line basis. Information available from such infrequent measurements is then subsequently used for monitoring of the bioprocess. Work in this area was first done by Halme *et al.* (1985). They used a Gaussian distribution function defined over a window around each sample point to quantify sample uncertainty. The component gains of the estimator related to the measurement were made to approach zero at the boundary of the window. Thus each sample had a validity interval after which it provided very little or no useful information to the estimator performance. In another approach, for antibiotic fermentations, Stone et al.(1992) used off-line measurements to update the maintenance coefficient that appeared in an inferential correlation relating the carbon dioxide evolution rate (CER) to the biomass. This updating used a balancing method of Esener *et al.* (1981) which was valid at low specific growth rates only and was performed off-line.

In this chapter, the problem of state and parameter estimation for a class of fermentations that exhibit significant endogenous metabolism and time varying maintenance activity is addressed. Occurrence of endogenous metabolism results in a decrease in the net specific growth rate of the microorganisms. The carbon dioxide evolution is however only due to the growth and maintenance processes that occur in the fermentation and as such does not reflect the effects of endogenous metabolism. Thus, there is an apparent loss of correlation between the CER and growth at the onset of endogenous metabolism. In addition, the true maintenance coefficient can also change during the fermentation. These problems are addressed by using a modified form of the traditionally used inferential relationship between the biomass growth and CER. Due to unobservability problems of some of the parameters in the modified relationship, the state and parameter estimation task cannot be performed by using the secondary measurements of CER alone as has been done in traditional approaches using the EKF. It has been shown that the infrequently available, delayed measurements of the biomass can also be formally accommodated in the estimation strategy to ensure good system observability and thus update all the parameters in the modified inferential relationship online. We also present experimental validation results of the resulting estimation strategy for monitoring biomass and the specific growth rate in a fed-batch fermentation of *Streptomyces clavuligerus* NRRL 3885

6.2 Materials and methods

Culture

The culture used in the experiments was *Streptomyces clauvuligerus* NRRL 3885, from which stock spores were prepared. Spores were scraped from TOA plates (oatmeal,20g/L; agar(Difco), 25g/L; pH=6.8) into 20 % glycerol. To speed up suspending, a sonicator was used. When the spore suspension was dark green, it was dispensed into 1.0ml cryovial and frozen at -70°C.

Inoculum culture was incubated in a 500ml shake flask containing 100ml medium. The composition of the medium[1] (per liter) was as follows : soluble starch(Difco), 10g; tryptone(Difco), 17g; soy peptone(Scott), 3g; NaCl 5g; K_2HPO_4, 1.25g; MOPS buffer, 20g; trace elements solution 1.0ml consisting of 0.1 % (w/v) each of $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$ and $CaCl_2$. The pH was adjusted to 6.8 with HCl. The medium was inoculated with 1%(v/v) spore suspension and grown on a rotary shaker (250 rpm) at 28°C for 24 hrs.

Equipment

All experiments were conducted using a 2L New Brunswick Multigen fermentor. Sensors for pH (Phoenix autoclavable double reference electrode),DO (Ingold autoclavable DO probe) and temperature (Omega) were connected to a microcomputer system via an OPTO-22 data acquisition system. The pH was controlled by an Omega PHCN-36 pH/ORP controller. A Tylan mass flow controller system coupled to the computer through the OPTO-22 system was used to regulate the flow of gas to the fermentor. The feeding rate was controlled by a Pharmacia peristaltic pump-1 through the computer. The fermentor off-gas was analyzed for concentrations of O_2 and CO_2 by a Dycor mass spectrometer.

Fermentation

All experiments were carried out with 2 % innoculum and an initial volume of medium of 1L. There were two feeding phases in all experiments. The first feeding phase started at a rate of 7.5 ml/h after 12 hrs of batch growth and finished at a rate of 22.5 ml/h after 24 hrs of feeding. The second feeding phase began almost immediately after the first and had a constant feeding rate of 3.5 ml/h until the end of the run(Figure 6.1)

The medium composition used in the batch growth phase was as follows: soluble starch, 3g/L; tryptone, 5.1g/L; soy peptone, 0.9g/L; NaCl, 5.0g/L; K_2HPO_4, 1.25 g/L; trace elements 1ml/L. The same medium was used in both the feeding phases and its composition was as follows : Maltose, 20g/L; Tryptone, 34g/L; soy peptone, 6g/L; NaCl, 5.0 g/L; K_2HPO_4, 1.25 g/L; trace elements, 1ml/L. During the fermentation, the pH was controlled between 6.7 and 6.9 with 2N HCl or 2N NaOH. The fermentor was agitated at 580 rpm, and aeration set point was 1.6 L/min. The foam was controlled using 2 % antifoam SAG 471 (Union Carbide).

Analyses

Samples taken from the fermentor were centrifuged to separate mycelia and the supernatant. The mycelia, after washing, resuspension and sonication, were used for protein analyses. The supernatant was analyzed for glucose, reducing sugar and starch. Proteins were determined using the dye-binding assay (Sedmack and Grossberg, 1977) on the supernatant obtained from the centrifugation of sonicated cells.

The Nelson-Somogyi micro-colorimetric method (Southgate, (1976)) was used to determine the total reducing sugar. Glucose concentration in the supernatant was determined using a glucose analyzer (YS1 Model 27). The maltose concentration was determined by taking the difference between the total reducing sugar and glucose concentrations.

Computational Methods

The computations for validating the estimation and filtering strategies were performed on MATLAB V4.0. (MATLAB, 1992).

6.3 Process Modelling

For fed-batch fermentation systems exhibiting endogenous metabolism, the following equation can be used to describe the net rate of growth of biomass,

$$\frac{dx}{dt} = \mu x - \mu_e x - \frac{q}{V}x \tag{6.1}$$

In the above equation, q is the nutrient feed rate, μ is the specific growth rate observed on substrate consumption and μ_e is the specific growth rate of the endogenous decay process (Roels (1983)). If we set $\mu_{net}=\mu-\mu_e$ and assume $x_1=xV$, where V is the volume of the broth, the above equation can be expressed more compactly as,

$$\frac{dx_1}{dt} = \mu_{net}x_1 \tag{6.2}$$

At constant pH and head pressure we can assume that the amount of CO_2 in the broth is approximately constant due to the rapid gas phase dynamics. Therefore, the total gaseous CO_2 outflow rate can be equated to the CER. The CER itself can be related, using a two parameter model, to the growth and non growth associated activities of the culture as,

$$CER = k_1 \mu \ x_1 + m x_1 \tag{6.3}$$

In the above equation, k_1 is the yield coefficient for CO_2 evolution on biomass growth and m is the maintenance coefficient. The above equation for CER can be written in terms of the net specific growth rate μ_{net} as :

$$CER = k_1 \mu_{net} x_1 + m_c x_1 \tag{6.4}$$

The coefficient m_c can be considered to be a modified maintenance coefficient representing the individual effects of the true maintenance and the endogenous decay processes. Comparing (1.3) and (1.4), the modified maintenance coefficient can be written as,

$$m_c = k_1 \mu_e + m \tag{6.5}$$

In typical fermentations, the specific growth rate μ is time varying due to its dependence on the time varying nutrient concentrations and therefore when the maintenance activity is negligible or constant, it is possible to use an EKF to simultaneously estimate the biomass as the state and the specific growth rate as the parameter from measurements of CER using equations (6.1) and (6.3). However, when there is significant endogenous metabolism and when the maintenance coefficient is not negligible or is time varying, the above equation set cannot be used to perform state and parameter estimation. This is because two of the parameters, μ_e and m are not observable from CER alone. Formulation of the modified equation set (6.2) and (6.4) helps in reducing the number of unobservable parameters by one. However, due to observability problems, it is still not possible to estimate both μ_{net} and m_c simultaneously from CER measurements alone. It is necessary to utilize information from other measurements which are sampled infrequently, such as the biomass to update the modified maintenance coefficient m_c.

Issues related to formal incorporation of the infrequently sampled measurement into the estimation equations for a Bayesian estimator have been investigated by the authors in an on-going research project on software sensor development. The authors have also investigated methods of enhancing system observability. We present here the estimation equations for the above problem.

Performing a simple Euler discretization on equation 6.2 we obtain,

$$x_1(i+1) = (1+\tau\mu(i))x_1(i) + w_1(i) \tag{6.6}$$

where τ is the sampling time and w_1 is the discretization error. A delay is usually associated with the sampling of the primary biomass measurement due to elaborate assay procedures. Assuming a delay of 2 sampling instants, we simulate them by introducing two hypothetical state variables as

$$x_2(i+1) = x_1(i) \tag{6.7}$$

$$x_3(i+1) = x_2(i) \tag{6.8}$$

The parameters μ_{net} and m_c can be modelled as random variables with unspecified distributions and can be written as,

$$\mu_{net}(i+1) = \mu_{net}(i) + w_2(i) \tag{6.9}$$

$$m_c(i+1) = m_c(i) + w_3(i) \tag{6.10}$$

The above time varying parameters can be updated along with the system states using an EKF formulation(Ray (1981), Stephanopoulos and San (1984)). For this task, an augmented system description is constructed by appending the parameters to the system state vector. The new augmented system description has a nonlinearity (bilinearity) due to terms that have a product of two states of the augmented state vector. State (and parameter) estimation is then carried out using the EKF by applying the linear Kalman filter equations to the linearized system. However, since we have multiple rates of sampling or measurement availability, the multirate formulation of the basic Kalman filter equations (Glasson (1983), Gudi *et al.* (1993)) needs to be used along with the linearized system description. For the above process description, it can be shown that the overall system of equations for the state vector $\underline{X}$ defined by $\underline{X}=[x_1;x_2;x_3;\mu;m_c]$ can be written as,

$$X(i+1) = \underline{f}[X(i)] + \underline{w}(i) \tag{6.11}$$

and the Jacobian associated with the linearization of the above equation is :

$$A = \begin{bmatrix} 1+\tau\mu(i) & 0 & 0 & \tau x_1(i) & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{6.12}$$

At any sampling instant i, we can have measurements of CER and/or the biomass. We term the sampling instant at which we have measurements of biomass and the CER as a major sampling instant. At the minor sampling instant, measurements of only the CER are assumed to be available. Using symbolic computation tools such as Maple (Waterloo Maple Software, (1981)), it can be shown that inclusion of as many past measurements of CER as the number of system delays enhances the observability properties of the new extended system. In addition, if an assumption is made that the parameters remain constant during the time period of delay, the system can be shown to be fully observable from the process outputs at the major as well as the minor sampling instants. It must be mentioned that the results of the above symbolic observability analysis are applicable in a generic but only a locally linearized sense to systems having a structure similar to the above system. Since we have assumed a delay of 2 sampling instants the measurement equation and the measurement vector at the major sampling instant can be expressed as,

$$h_{major} = \begin{bmatrix} x_3(i) \\ (k_1\mu_{net}(i)+m_c(i))x_1(i) \\ (k_1\mu_{net}(i)+m_c(i))x_2(i) \\ (k_1\mu_{net}(i)+m_c(i))x_3(i) \end{bmatrix} \tag{6.13}$$

$$z_{major} = \begin{bmatrix} x_3(i) \\ CER(i) \\ CER(i-1) \\ CER(i-2) \end{bmatrix} + v_{major} \tag{6.14}$$

where v_{major} is the associated measurement noise vector. At the minor sampling instants, the measurement equation and the measurement vector is :

$$h_{minor} = \begin{bmatrix} (k_1\mu_{net}(i)+m_c(i))x_1(i) \\ (k_1\mu_{net}(i)+m_c(i))x_2(i) \\ k_1\mu_{net}(i)+m_c(i))x_3(i) \end{bmatrix} \tag{6.15}$$

$$z_{minor} = \begin{bmatrix} CER(i) \\ CER(i-1) \\ CER(i-2) \end{bmatrix} + v_{minor} \tag{6.16}$$

The linearized measurement equations at the major and minor sampling instants are:

$$H_{major} = \frac{\partial h_{major}}{\partial X} = \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ k_1\mu(i)+m_c i & 0 & 0 & k_1x_1(i) & x_1(i) \\ 0 & k_1\mu(i)+m_c(i) & 0 & k_1x_2(i) & x_2(i) \\ 0 & 0 & k_1\mu(i)+m_c(i) & k_1x_3(i) & x_3(i) \end{bmatrix} \tag{6.17}$$

$$H_{minor} = \frac{\partial h_{minor}}{\partial X} = \begin{bmatrix} k_1\mu(i)+m_c(i) & 0 & 0 & k_1x_1(i) & x_1(i) \\ 0 & k_1\mu(i)+m_c(i) & 0 & k_1x_2(i) & x_2(i) \\ 0 & 0 & (k_1\mu(i)+m_c(i) & k_1x_3(i) & x_3(i) \end{bmatrix} \tag{6.18}$$

The time varying formulation of the basic Kalman filter(Franklin *et al.*,(1990) modified for the case of multirate measurements (Gudi *et al.*,(1993)) can now be used alongwith the above linearized system of equations to set up the estimator algorithm.

Assuming that a state prediction $\bar{X}$ and the state covariance prediction M are available at the major sampling instant, the innovations ε_{major} is first generated in equation (6.19) using the measurements. The linearized Kalman gain K_{major} is then calculated in equation (6.20) using the measurement equation linearized around the current state prediction $\bar{X}$. The state estimate $\hat{X}$ is then generated by suitably weighing the innovations and the state prediction in equation (6.21). The *aposteriori* (after measurement) state covariance matrix P, which represents the estimation accuracy immediately after the measurement is processed, is then calculated in equation (6.22). P is then used in equation (6.28) to generate a prediction of the state covariance for the next sampling instant.

Thus at the major sampling instant, the estimation equations are written as,

Major Sampling instant

$$\varepsilon_{major} = z_{major} - h_{major} \tag{6.19}$$

$$K_{major} = MH^T_{major}[\underline{\bar{X}}(i)]\ \{H_{major}[\underline{\bar{X}}(i)]M(i)H^T_{major}[\underline{\bar{X}}(i)] + R_{z,major}\}^{-1} \tag{6.20}$$

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{major}\underline{\varepsilon}(i) \tag{6.21}$$

$$P(i) = M(i) - K_{major}h_{major}M(i) \tag{6.22}$$

At the minor sampling instant, the above set of equations can be rewritten with different (smaller) dimensions on the measurement equations and the Kalman gains. The equations at the minor sampling instant can be written as,

Minor Sampling instant

$$\varepsilon_{minor}(i) = z_{minor} - h_{minor} \tag{6.23}$$

$$K_{minor} = MH^{T}_{minor}[\underline{\bar{X}}(i)]\{H_{minor}[\underline{\bar{X}}(i)]M(i)H^{T}_{minor}[\underline{\bar{X}}(i)] + R_{z,minor}\}^{-1} \tag{6.24}$$

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{minor}\underline{\varepsilon}(i) \tag{6.25}$$

$$P(i) = M(i) - K_{minor}h_{minor}M(i) \tag{6.26}$$

In the above equations, R_z is the covariance of measurement noise and is chosen to reflect the errors in the measurement device. The above equations are essentially the measurement update equations at any instant i assuming that the state prediction $\bar{X}$ and covariance prediction M are available. The latter are provided by the time update equations which are written by performing an actual integration of the nonlinear equation set as,

$$\underline{\dot{X}} = \underline{f}\,(\underline{\hat{X}},t) \tag{6.27}$$

$$\dot{M} = \hat{f}_X\, P + P\, f_X^T + Q \tag{6.28}$$

In the above equations, Q is the covariance of process noise and is chosen to reflect errors in the process model. The iterated extended Kalman filter (IEKF) has been recommended for systems with nonlinearities in the measurement equation (Bellgardt *et al.* (1986)). The authors have investigated the possibility of using the IEKF in the above multirate framework. However, the nonlinearities in the above measurement equations were not found to significantly influence the convergence characteristics of the multirate estimator. The above equations were thus found to be adequate to perform state and parameter estimation tasks at the major and minor sampling instants.

6.4 Results and Discussion

Fermentation

The fermentation experiments were fairly reproducible and a single experiment which was found to be representative of nine experiments was chosen for the estimator validation. Figure 6.1 shows the profiles for the cumulative biomass, the feeding policy and the carbon dioxide evolution rate. The maltose concentrations were between 1-2 g/L throughout the fermentation run. The culture exhibited a lysis phase at or about 40 h of fermentation. This phenomenon was verified from the profiles of dry cell weight as well as the protein content and was observed for both the fed-batch and batch runs. Since the maltose concentrations were fairly steady, the onset of lysis indicated a limitation of some other critical nutrient necessary for growth.

Estimator Performance

Estimator validation was carried out by presenting the cumulative biomass (with an assumed measurement delay) and the CER data in an "on-line" fashion to the estimation equations. The estimator was run on the basis that the time period between two sampling instants (basic sampling time) was 20 minutes. Accordingly, the major sampling instants arrived approximately every nine sampling instants (3 h) for the sampling strategy chosen. At this sampling instant, measurements of both , the cumulative biomass (possibly delayed i.e the measurement corresponded to sampling done at an earlier time) and the CER, were available. At the minor sampling instants, which occurred after every basic sample time, measurements of only the CER were available. It was proposed to generate estimates for the cumulative biomass, specific growth rate and the modified maintenance coefficient from the data collected for the fed-batch fermentation. k_1 has been assumed to be 0.0372 mmol CO_2 / g X (Pirt *et al.*(1967), Stone *et al.* (1992)). The initial values for the states and parameters assumed for the estimation run are presented in Table 1.

Reference parameter profiles

The estimator was first run by assuming that the cumulative biomass and the CER measurements were available at every sampling instant. The cumulative biomass values

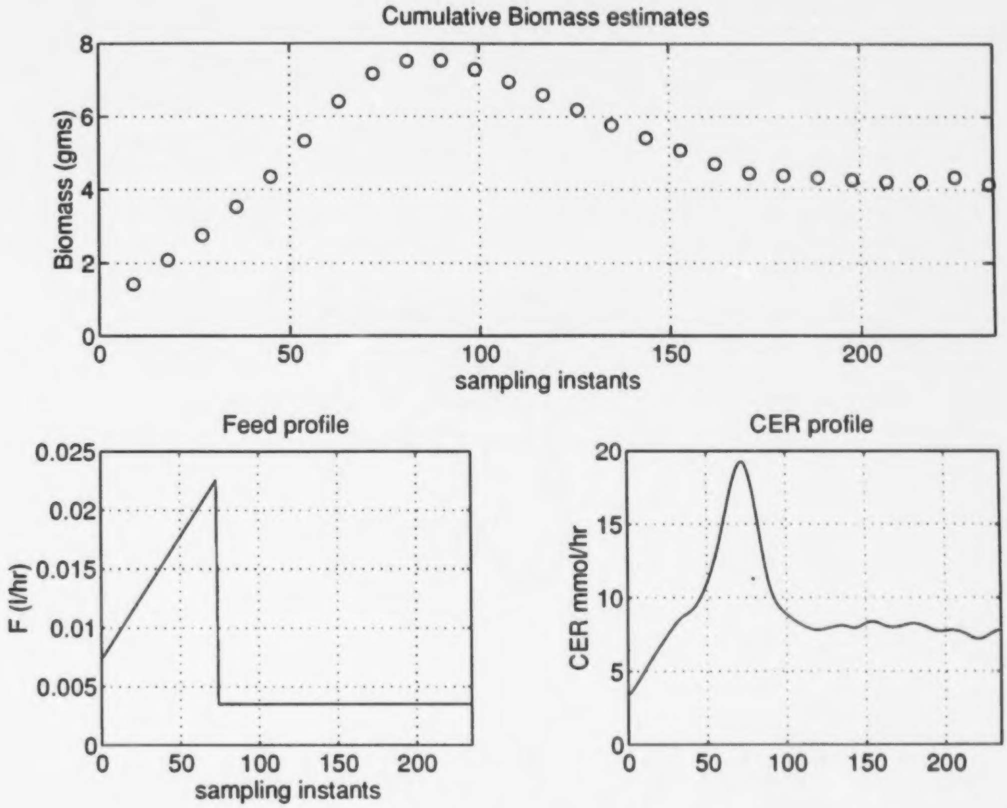


Figure 6.1: Experimental profiles for the cumulative biomass, the nutrient feeding rate and the CER for fed-batch feremetation of *Streptomyces clavuligerus* NRRL 3885. A single fermentation run representative of nine runs is shown. The biomass measurements were taken every 3 h and the CER measurements were taken every 20 minutes.

that were needed for such a run were generated by linear interpolation of the sampled points. Under such a scenario, all the sampling instants were major ones, and therefore the system was completely observable at all the sampling instants. The parameter profiles that were generated converged to values that could be verified from visual examination of the biomass and CER profiles. At the end of the fermentation, the specific growth rate was close to zero. Thus all the CER could be attributed to maintenance activity alone. Knowing the terminal biomass and CER values, it is possible to verify that the expected terminal value of the maintenance coefficient was about 1.8 mmol CO_2/hr-g.biomass. This value was in close agreement with what was predicted from by the estimator. Thus, the reference parameter trajectories generated above were assumed to be representative of the true values in the fermentation and were then used to compare the performance of the estimator under a multirate sampling scenario.

Effect of measurement delay

Figure 6.2 presents the results of the estimation for different values of the measurement delay. For the case when no past CER measurements are included in the measurement set, the system observability matrix is rank deficient at the minor sampling instant. The maintenance coefficient is not observable from the instantaneous CER measurement. The system is, however, fully observable at the major sampling instant and accordingly, the maintenance coefficient is updated only at the major sampling instants and remains constant at the minor sampling ones. With a progressive increase in measurement delay from two to four sampling instants(40 min to 80 min), the rank deficiency of the observability matrix at the minor sampling instant increases, because the additional states that need to be incorporated in the system description to simulate measurement delays, are also now unobservable from the system output. This enabled the updating of maintenance coefficient to be performed only at the major sampling instants and hence its tracking was sluggish.This resulted in biased state and parameter estimates.

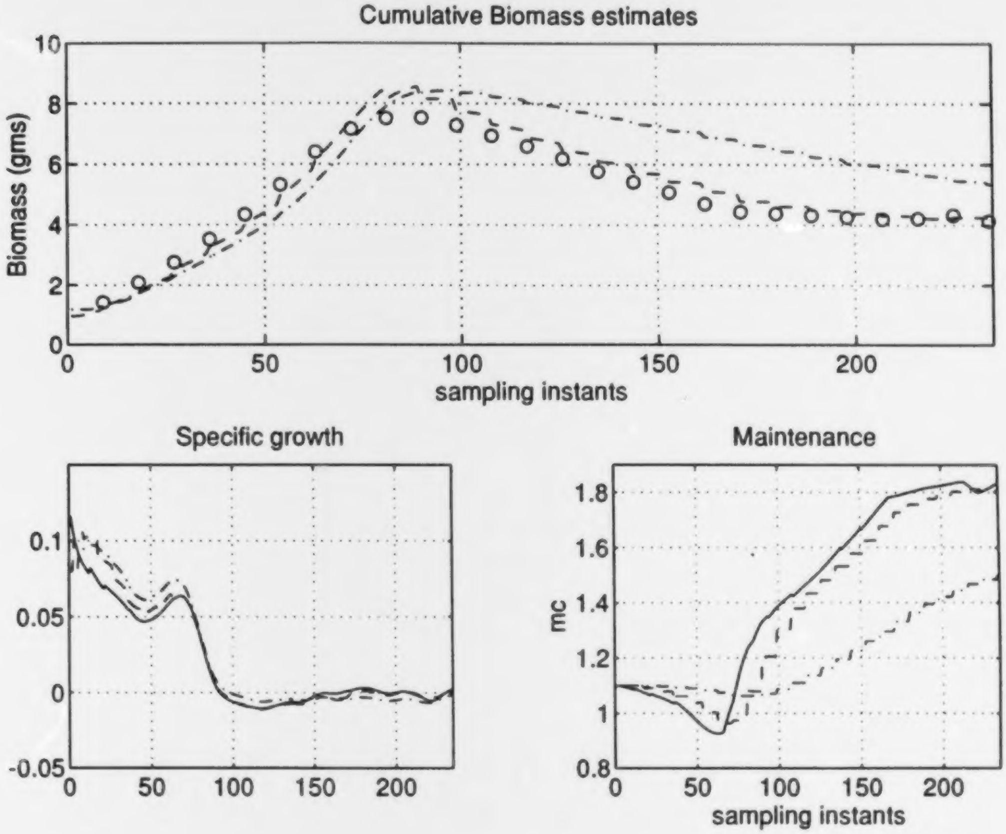


Figure 6.2 : Estimator performance in the presence of measurement delay using instantaneous CER values. (o) Observed experimental values for biomass (g);(--) estimated profiles assuming a measurement delay of 40 min; (-.) estimated profiles assuming a measurement delay of 80 min;(solid line) reference parameter profiles.

Modified observer performance

In the modified measurement equation, the unobservability of the delayed states at the minor sampling instants is addressed by appending as many past measurements as the number of delayed states. Furthermore, making an assumption that the parameters remain constant during the measurement delay time, makes the system fully observable at the minor sampling instants too. Thus, incorporation of the modified measurement equations (13-16) makes the system fully observable at all sampling instants. Figure 6.3 shows the performance of the estimator when the modified measurement equations are incorporated. It can be seen that the estimator performance about the time the feed is switched to a smaller value (after 24 h of linearly increasing feed) was a little erratic. This is because the assumption made regarding the constancy of parameters during the measurement delay time was not valid. The true parameters changed quite rapidly during this time due to the transient response of the culture to the feed change. However, the parameter estimates did converge quickly to the reference values; this is a result of improved system observability realized by incorporation of the modified measurement equations. The estimator can be seen to be quite robust to measurement delays. An increase in the measurement delay from 40 minutes to 80 minutes does not have any significant effect on the estimated parameter profiles. If a more rapid sampling scheme is chosen in the transient behaviour region, the state and parameter tracking are improved. This can be seen in Figure 6.4, when the major sampling instants arrive after every 40 minutes. Thus, the multirate estimator is capable of providing unbiased state and parameter estimates for the case of multirate sampling with measurement delay.

6.5 Interpretation of Results

Significant maintenance and endogenous metabolism related activity can be expected during the nutrient limitation phase resulting from a shift in the nutrient feeding policy. Recognising this, Mou *et al.* (1983) used a different correlation during the transition and penicillin production periods, to calculate cell growth. The parameter m_c defined in this

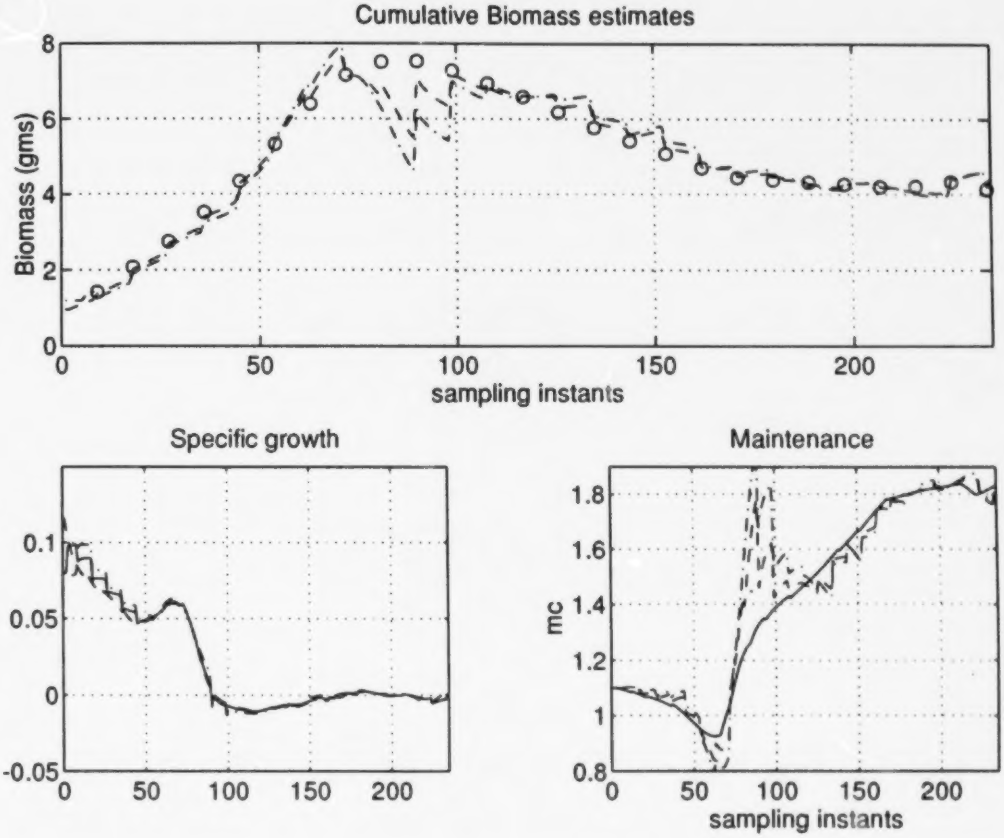


Figure 6.3 : Estimator performance in the presence of measurement delay using modified measurement equation. (o) Observed experimental values for biomass (g);(--) estimated profiles assuming a measurement delay of 40 min; (-.) estimated profiles assuming a measurement delay of 80 min;(solid line) reference parameter profiles.

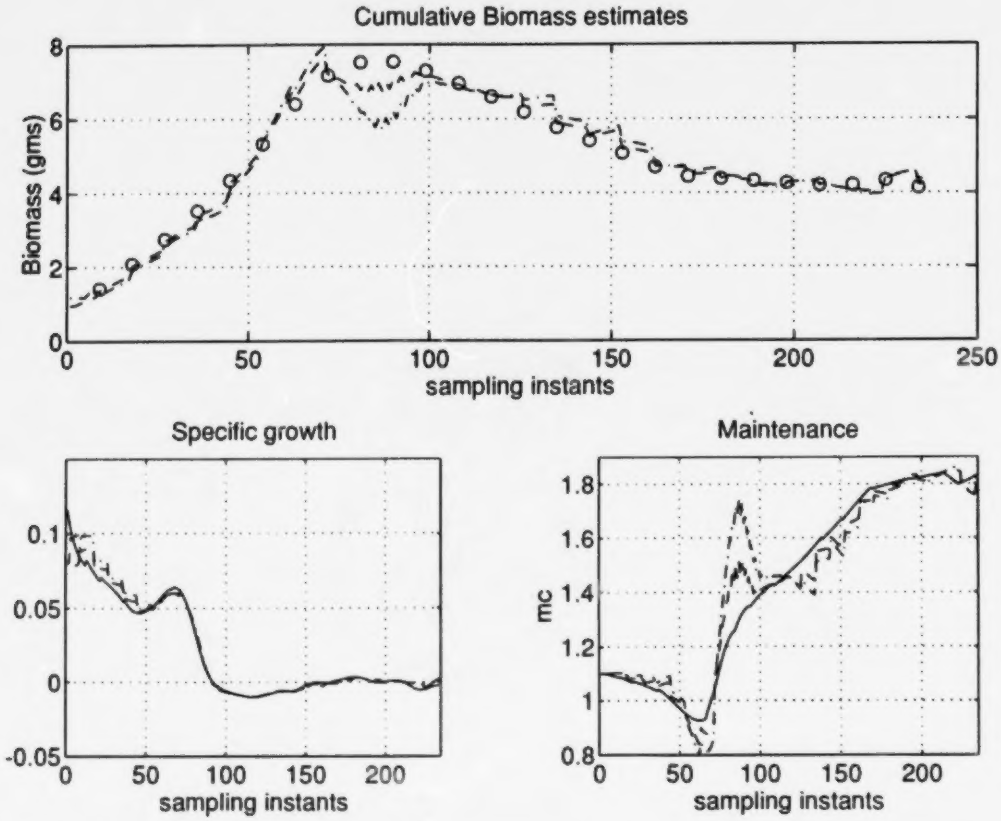


Figure 6.4: Estimator performance in presence of measurement delay using modified sampling scheme in the transient region. Biomass values needed for the modified sampling scheme were obtained by linear interpolation. (o) observed experimental values for biomass (g);(--) estimated profiles assuming a measurement delay of 40 minutes; (-.) estimated profiles assuming a measurement delay of 80 minutes;(solid line) reference parameter profiles.

study includes the effect of endogenous metabolism and the true maintenance activity. For the fermentation studied here, the sudden switching over from a linearly increasing nutrient feed rate to a smaller constant value could have caused a limitation in some key component necessary for growth. This limitation could have triggered the endogenous decay process, causing μ_e in equation (6.1) to assume a small positive value. This interpretation could explain in part the increase in the value of m_c during the later time of fermentation.

The true maintenance coefficient m can also change during the fermentation. As has been shown by Niejssel *et al.* (1975), the true maintenance rate can be manipulated by the rate at which the carbon substrate is added to the culture. They observed that it is also a function of the redox state of other essential nutrients. Hill *et al.* (1993) have also observed an increase in the combined maintenance and energy spilling or wastage components under conditions of nutrient limitation. For the fermentation studied here, it is also possible that the microorganism had to expend more energy to assimilate the nutrients available from the endogenous decay process, thus resulting in an increase in the true maintenance energy during the lysis phase of the fermentation.

6.6 Conclusions

Problems associated with estimation and monitoring of fermentation processes that exhibit significant endogenous metabolism and time-varying maintenance activity have been addressed. Modified model equations have been proposed and used along with a multirate state and parameter estimator that can formally accommodate the delayed, infrequent primary measurement. Experimental validation studies of the resulting software sensor, for the estimation of states and parameters in a fed-batch fermentation of *Streptomyces clavuligerus*, have been successfully carried out.

6.6 References

Aharonowitz, Y., Demain, A.L. Carbon catabolite regulation of cephalosporin production in *Streptomyces clavuligerus* , Antimicrob. Agents. Chemother.,**14**:159-164, (1978).

Bastin, G., Dochain, D. Online estimation and adaptive control of Bioreactors, Elsevier Science Publishing Co. Inc, Amsterdam, Netherlands, (1990).

Bellgardt, K., Kuhlmann, W., Meyer, H., Schugerl, K. and Thoma, M. Application of an extended Kalman filter for state estimation of a yeast fermentation. IEE proceedings, **133**(5): 226-234, (1986).

Esener, A.A., Roels, J.A., Kossen N.W.F. Fed-batch Culture : Modelling and Applications in the study of Microbial Energetics, Biotechnology and Bioengineering, **23**:1851-1871, (1981).

Franklin, G.F., Powell, J.D., Workman, M.L. Digital Control of Dynamic systems, Addison Wesley Publishing Inc, Reading, Massachussets, USA, (1990).

Glasson, D.P. Development and Applications of Multirate Digital Control , IEEE Control Systems Magazine,**3**(4):2-8,(1983).

Gudi, R.D., Gray, M.R.,Shah, S.L. Multirate estimation and monitoring of Process variables in a bioreactor, p487-492, In: Proc. 2nd IEEE conference on Control Applications, IEEE Control Systems Society, New York, (1993).

Halme, A.,Kuismin, R.,Koteniemi, M. A method to consider delayed laboratory analysis in state and parameter estimation of Bioreactors, p199-204, In: A Johnson (Ed) Proc. of 1st IFAC symposium on Modelling and Control of Bioprocesses, Pergamon, Oxford, U.K, (1985).

Herbert, D., Some principles of continous culture, Recent Progress in Microbiology,**7**:381-396,(1958).

Hill, P.W., Klapatch, T.R., Lynd, L.R. Bioenergetics and End-Product Regulation of *Clostridium thermosaccharolyticum* in response to nutrient limitation. Biotechnology and Bioengineering, **42**:873-883, (1993).

Waterloo Maple Software, 450 Phillip St., Waterloo, Ontario, Canada N2L 5J2, (1981).

MATLAB, The Mathworks Inc., 24 Prime Park Way, Natick, MA 01760, USA, (1992).

Mou,D.G., Cooney, C.L. Growth Monitoring and Control through Computer-aided on-line mass balancing in a fed-batch Penicillin fermentation, Biotechnology and Bioengineering, **25**:225-255, (1983).

Neijssel, O.M.,and Tempest, D.W. The regulation of carbohydrate metabolism in Klebsiella aerogenes NCTC 418 organisms, growing in continous cultures. Arch. Microbiol.,**106**:251-288, (1975).

Pirt, S.J. The maintenance energy of Bacteria in growing cultures. Proc. R. Soc Lond. Ser. B **163**:224-231, (1965).

Pirt, S.J., and Righelato, R.C. Effect of growth on the synthesis of penicillin by *Penicillium chrysogenum* in batch and chemostat cultures, Applied Microbiology, **15**:1284-1289, (1965).

Ray, W.H. Advanced Process Control. McGraw-Hill Book Company, New York, USA, (1981).

Roels, J.A. Energetics and Kinetics in Biotechnology. Elsevier Biomedical Press, Amsterdam, Netherlands, (1983).

Sedmack, J.J., Grossberg, S.E. A rapid sensitive and versatile assay for protein using coomassie brilliant blue G250. Analytical Biochemistry. **79**:544-552, (1977).

Southgate, D.A.T. Determination of food carbohydrates,P105-106, Applied Science Publishers Ltd., London, U.K., (1976).

Stephanopoulos, G. and San, K.Y. Studies on on-line bioreactor identification, Biotechnology and Bioengineering.,**26**:1176-1188, (1984).

Stone, K.M, Thornhill, N.F, Roche, F.W.,Fish, N.M. A method of using off-line measurements in an on-line estimator of biomass concentration for a penicillin fermentation and its effects on the quality of the estimates, p101-107, In : Karim M.N, Stephanopoulos G., (Eds), Modelling and Control of Biotechnical Processes 1992, Proceedings of 2nd IFAC symposium on Modelling and Control and 5th International Conference on Control Applications to Fermentation Technology, Pergammon , Oxford, U.K., (1992).

6.7 Nomenclature

f	nonlinear model of the augmented state
h	measurement equation at any sampling instant
k_1	yield coefficient for CO_2 evolution over biomass growth
m	maintenance coefficient in terms of mmol CO_2/hr-1
m_c	modified maintenance coefficient
q	nutrient feeding rate for the fed-batch fermentation
t	time variable
x	cumulative biomass in mass units
z	measurement vector in sampling instant
A	jacobian associated with the linearization
H	linearized measurement equation at any sampling instant
M	*a priori* estimate of the covariance of estimation errors
Q	covariance of process noise
R	covariance of measurement noise
V	fermentation broth volume

Greek

μ	specific growth rate
μ_e	specific growth rate of the endogenous decay process
μ_{net}	specific growth rate
ε	innovations vector

Subscripts

major	pertaining to major sampling instant
minor	pertaining to minor sampling instant

Superscripts

^	state estimate via measurement update
-	state prediction via time update

TABLE 6.1

Initial conditions and parameters used for the estimation runs

Initial Conditions

$x_1(0), x_2(0), \ldots, x_{ndelay}(0) = 0.9596$ g

$\mu(0) = 0.08$ hr^{-1}

$m_c(0) = 1.1$ mmol CO_2/g X-hr

Parameters

Yield coefficient $k_1 = 0.0372$ mmol CO_2/g X

Covariance matrix Q= diagonal matrix of appropriate size having values 10 for the states and 30 for parameters

Covariance matrix R = diagonal matrix of appropriate size with values 0.00105 as diagonal elements.

Chapter 7

Adaptive Multirate Estimation and Control of Nutrient Levels in a Fed-Batch fermentation using off-line and on-line Measurements[1]

A multirate adaptive estimation algorithm developed earlier (Gudi *et al.* (1995)) is extended to perform estimation of nutrient levels using frequent on-line measurements of the carbon dioxide evolution rate(CER) and off-line, infrequent and delayed measurements of the biomass and the substrate concentrations. It has been shown that the algorithm can be designed to track changing substrate yield coefficients as well. The estimation algorithm has been verified using simulations and industrial data from a fed-batch fermentation involving a secondary metabolite producing microorganism. It has been coupled with a nonlinear control law designed to track prespecified optimal nutrient trajectories. The resulting closed loop control scheme is evaluated using simulation runs.

---

[1] A version of this chapter has been submitted for publication to the Canadian Journal of Chemical Engineering as: R.D. Gudi, S.L. Shah, M.R. Gray and P.K. Yegneswaran, " Multirate Estimation and Control of Nutrient levels in a fed-batch fermentation using off-line and on-line Measurements".

Chapter 7: Multirate estimation and Control of Nutrient Levels.

7.1 Introduction

This chapter focuses on estimation and control of nutrient levels in a fed-batch fermentation. From a control perspective, the nutrient or substrate level in a fed-batch fermentation is the most important state variable that influences the growth and product expression reactions. However, due to a lack of adequate on-line sensors, it is difficult to measure the nutrient concentrations at a desired sampling rate. Often a measurement delay is also associated with the nutrient measurements due to elaborate off-line assay procedures. These problems make the regulation of nutrient levels a challenging task.

Strategies to inferentially generate estimates of unmeasured state variables, such as nutrient concentration, from measurements of other secondary variables have been proposed to address this problem. Stephanopoulos and San (1984) used an extended Kalman filter (EKF) to generate estimates of critical culture states such as the biomass and the substrate concentrations and parameters such as the specific growth rate from measurements of exit gas concentrations. To be able to perform the above estimation, a system related mathematical criterion called observability needs to be ensured. Loosely speaking, a system is said to be observable if all its states can be estimated from a knowledge of the model and some measurements of a linear combination of a small subset of the states. The estimator model used by Stephanopoulos and San (1984) did not allow the observation of nutrient concentrations from exit gas CO_2 measurements. They therefore proposed to first generate estimates of the observable states and parameters from exit gas measurements, then substitute these values into a dynamic nutrient balance equation which could be integrated over time to yield estimates of the nutrient levels. Although this strategy works quite well, the nutrient estimation strategy is open loop. Park and Ramirez (1990) used a sequential parameter updating strategy to monitor and regulate nutrient levels at desired values. However, they assumed that the nutrient measurements were available at desired sampling rates.

In general, sensors to measure nutrient levels on-line at regular intervals, without a time delay for analysis do not exist. In addition, unless relationship expressions such as the Monod or Contois models that relate the specific growth rate to nutrient levels are assumed, the nutrient concentrations are unobservable from other growth rate related measurements. The yield coefficient of substrate on biomass may also be typically unknown and can possibly change. Stephanopoulos and San (1984) used material balances based on molecular reactions and an assumed stoichiometric formula for the biomass. This method generates estimates of the yield coefficient; however, the stoichiometric balances are not easy to write for complex fermentations. An incorrect estimate of the yield coefficient can result in biased estimates of the nutrient levels.

In this chapter, we propose a novel approach to address these problems. The observation strategy that we propose is based on formal incorporation of the infrequently available and delayed measurements of the primary variables such as the biomass and substrate into the estimation strategy. In an earlier work (Gudi *et al.*,1995), we have shown that a Kalman filter expressed in a multirate framework coupled with an estimator model that describes delays, can be used to perform the tasks of filtering and estimation when measurements are available at different time scales and with delays. This estimator has been successfully used to generate estimates of the biomass and the specific growth rates from on-line exit gas and off-line, delayed biomass measurements. This approach requires the states and parameters in the estimator model to be fully observable from the measurements available at any sampling instant. Since models proposed in the literature to relate the specific growth rate with nutrient levels have problems in estimation of their parameters(Bastin and Dochain,1990), they are not commonly used. Rather, the time evolution of the specific growth rate is modelled as a white noise process. Hence the nutrient levels are unobservable from the on-line, growth related, exit gas measurements. Thus, the nutrient levels cannot be updated at instants when only exit gas measurements are available. To address this problem, we propose to cascade a multirate estimator for biomass and specific growth rate estimation (Gudi *et al.*,1995) with another estimator designed to estimate nutrient levels. At sampling instants when only exit gas measurements are available, the nutrient estimator uses the biomass and specific growth rate estimates to update its model. It then generates estimates of the nutrient levels by direct integration of the model equations. In this sense, at these sampling instants, the observation strategy is similar to that used by Stephanopoulos and San(1984). However, the nutrient estimator also formally accommodates the nutrient measurement whenever the latter is available. A structural observability analysis has been performed and presented to show that it is possible to estimate and update the substrate over biomass yield coefficient at instants when a delayed nutrient measurement is available. It is shown that the infrequently available, delayed measurement of the nutrient concentration provides feedback information and corrects the estimates generated inferentially by integrating the appropriately updated nutrient balance equation, thus evolving a closed-loop observation strategy. The new estimation algorithm has been validated using simulations involving a fed-batch fermentation. It has also been successfully evaluated using data from an industrial antibiotic fermentation involving a secondary metabolite producing specie. The estimation strategy has also been coupled with a nonlinear control law designed to regulate/track nutrient levels. The resulting closed loop control algorithm is validated by simulations involving a fed-batch fermentation process.

7.2 Process model

The dynamic mass balances for biomass growth and substrate depletion in a fed batch fermentation can be written as,

$$\frac{dx}{dt} = \mu x - \frac{F}{V} x \tag{7.1}$$

$$\frac{ds}{dt} = -k_s \mu x + \frac{F}{V}(s_{in} - s) \tag{7.2}$$

$$\frac{dV}{dt} = F \tag{7.3}$$

In the above equations, x is the biomass concentration, s is the nutrient concentration,V is the broth volume and F is the nutrient feeding rate. Performing simple variable transformations $x_1 = xV$ and $s_1 = sV$ and then discretizing using a simple Euler discretization method for a basic sampling time τ, the above equations can be rewritten at any sampling instant i as,

$$x_1(i+1) = (1+\tau\mu(i))x_1(i) + w_x(i) \tag{7.4}$$

$$s_1(i+1) = -k_s(i)\tau\mu(i)x_1(i) + s_1(i) + Fs_{in} + w_s(i) \tag{7.5}$$

where μ is the specific growth rate of the biomass. It is time variant and a function of several physio-chemical and biological factors. Several growth models relating μ to the culture states have been proposed (Bastin and Dochain, 1990). However, there are practical difficulties in identifying the kinetic constants of such a model and it is therefore recommended by Bastin and Dochain (1990) that μ be treated as a time varying parameter which can be estimated online. The time evolution of μ is therefore modelled as a white noise process of unknown probability density. Thus,

$$\mu(i+1) = \mu(i) + w_1 \tag{7.6}$$

It must be noted that treating μ as an independent time varying parameter however masks the link between substrate depletion and other growth related activities of the culture, thus making the nutrient concentrations unobservable from the growth related measurements. k_s is the yield coefficient of substrate on biomass. It could be typically unknown or time varying and is also modelled as a white noise process,

$$k_s(i+1) = k_s(i) + w_2 \tag{7.7}$$

The process outputs of primary interest are the substrate and biomass concentrations which as mentioned before are measured infrequently and with some delay. We term these variables as the primary variables. Other secondary process outputs such as the carbon dioxide evolution rate (CER) can be obtained online more frequently and without much delay. These secondary process outputs are correlated with the primary process outputs and can be used in an inferential setup to estimate the latter, provided that certain systems related criterion such as observability is met. The CER can be related to the growth and maintenance related activity of the culture (Gudi *et al.*,1994) as,

$$CER(i) = k_1 \mu(i) x(i) + m_c(i) x(i) \tag{7.8}$$

In the above equation m_c is the maintenance coefficient which is typically time varying for the class of secondary metabolite producing fermentations. It can be modelled by a white noise process and can be described as,

$$m_c(i+1) = m_c(i) + w_3 \tag{7.9}$$

Delays in measurement availability of the primary variables result in decreased system observability. For simplicity, let us assume a measurement delay of two sampling instants in the biomass and substrate measurements. The delays can then be included in the estimator model by the addition of fictitious delayed states as,

$$x_2(i+1) = x_1(i) \tag{7.10}$$

$$x_3(i+1) = x_2(i) \tag{7.11}$$

$$s_2(i+1) = s_1(i) \tag{7.12}$$

$$s_3(i+1) = s_2(i) \tag{7.13}$$

Our objective is to generate estimates of the states x_1, x_2, x_3, s_1, s_2, s_3 and the parameters k_s, μ and m_c from on-line inferential measurements of the secondary variables and off-line delayed primary variable measurements. We call the sampling instants at which we have the delayed primary measurement and the current secondary measurement as a major sampling instant. Thus, at the major sampling instants, the measurement vector and the measurement equation can be written as,

$$z_{major} = \begin{bmatrix} x_3(i) \\ s_3(i) \\ CER(i) \end{bmatrix} + v_{major} \tag{7.14}$$

$$G_{major} = \begin{bmatrix} x_3(i) \\ s_3(i) \\ (k_1\mu(i) + m_c(i))x_1(i) \end{bmatrix} \tag{7.15}$$

At the minor sampling instant, only the current secondary measurements are assumed to be available and accordingly the measurement vector and the measurement equation can be written as,

$$z_{minor} = CER(i) + \nu_{minor} = (k_1\mu(i)+m_c(i))x_1(i) + \nu_{minor} \tag{7.16}$$

$$G_{minor} = (k_1\mu(i) + m_c(i))x_1(i) \tag{7.17}$$

Two issues need to be addressed related to the above objective of estimating the states and parameters. Firstly, we need to perform the task of estimation when we have a multirate sampling scenario i.e. the slow availability of the primary measurements and the relatively fast availability of the secondary measurements. Simultaneously, we also need to address the issue of decreased system observability due to delays in the measurement of the primary variables.

7.3 Estimator equations

To satisfy the first objective of filtering and estimation in the presence of multirate measurements, a multirate Kalman filter (Glasson,(1980),(1983)) based estimation scheme has been shown (Gudi *et al.*(1993a,b)) to formally accommodate the slowly sampled primary measurement into the observation strategy. In the presence of time varying parameters however, an extended version of the multirate Kalman filter needs to be used. In addition, Gudi *et al.*(1995) have shown that weak system observability due to measurement delays in the primary variable measurements can be enhanced or compensated by appending as many past inferential measurements as the number of delays. We have also shown, based on a structural observability analysis, that it is possible to obtain relatively "strong" system observability when past inferential measurements are appended to the measurement vector at the major sampling instants as well. These ideas can be used here to generate estimates of the state x_1,x_2,x_3,s_1,s_2,s_3 and the parameters k_s,μ as follows.

Due to the assumption that the specific growth rate μ is independent of the nutrient concentrations, the latter are unobservable from the CER measurements. Therefore, we estimate the biomass states and the nutrient states separately. Consider first the sub-problem of estimating the biomass states and the parameters μ and m_c from the off-line biomass and regular CER measurements. As shown in Gudi *et al*(1994), the following estimator equations give unbiased state and parameter estimates.

Estimator I: The states in this estimator are given by $X=[x_1\ x_2\ x_3\ \mu\ m_c]$ and the relevant system equations are those given by equations (7.4),(7.6),(7.9 - 7.11). At the major sampling instants, the measurement vector and the measurement equation are given by,

$$z_{major,I} = \begin{bmatrix} x_3(i) \\ CER(i) \\ CER(i-1) \\ CER(i-2) \end{bmatrix} + v_{major,I} \tag{7.18}$$

$$G_{major,I} = \begin{bmatrix} x_3(i) \\ (k_1\mu(i)+m_c(i))x_1(i) \\ (k_1\mu(i)+m_c(i))x_2(i) \\ (k_1\mu(i)+m_c(i))x_3(i) \end{bmatrix} \quad . \tag{7.19}$$

Assuming that the linearized measurement matrix is given by $H_{major,I}=\partial G_{major,I}/\partial X$ and the *a priori* covariance of the estimation error is given by M_I, the measurement update equations, for the time varying Kalman filter (Franklin *et al.* (1990)) formulation, are :

a) Major sampling instant

(i) Measurement update :

$$K_{major,I} = M_I\, H^T_{major,I}(H_{major,I} M_I H^T_{major,I} + R_{z,major,I})^{-1} \tag{7.20}$$

$$\varepsilon_{major,I}(i) = z_{major,I} - G_{major,I} \tag{7.21}$$

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{major,I}\,\varepsilon_{major,I}(i) \tag{7.22}$$

$$P_l(i) = M_l(i) - K_{major,l} H_{major,l} M_l(i) \tag{7.23}$$

At the minor sampling instants, the measurement vector and the measurement equation are :

$$z_{minor,l} = \begin{bmatrix} CER(i) \\ CER(i-1) \\ CER(i-2) \end{bmatrix} + v_{minor,l} \tag{7.24}$$

$$G_{minor,l} = \begin{bmatrix} (k_1\mu(i) + m_c(i))x_1(i) \\ (k_1\mu(i) + m_c(i))x_2(i) \\ (k_1\mu(i) + m_c(i))x_3(i) \end{bmatrix} \tag{7.25}$$

The measurement update equations at the minor sampling instants are given by :

b) Minor sampling instant

(i) Measurement update :

$$K_{minor,l} = M_l\, H^T_{minor,l}(H_{minor,l} M_l H^T_{minor,l} + R_{z,minor,l})^{-1} \tag{7.26}$$

$$\varepsilon_{minor,l}(i) = z_{minor,l} - G_{minor,l} \tag{7.27}$$

$$\underline{\hat{X}}(i) = \underline{\bar{X}}(i) + K_{minor,l}\varepsilon_{minor,l}(i) \tag{7.28}$$

$$P_l(i) = M_l(i) - K_{minor,l} H_{minor,l} M_l(i) \tag{7.29}$$

The state predictions needed to perform the measurement updates are obtained by performing an actual integration of the relevant system equations. The prediction of the a priori covariance of the estimation error is obtained by performing an integration of the following equation :

$$M_l(i+1) = F_X P_l(i) F_X^T + Q_l \tag{7.30}$$

where F_X is the Jacobian of the system description. Q_l is the covariance matrix of process noise and $R_{z,major,l}$ and $R_{z,minor,l}$ are the covariance matrices of the measurement noise at the major and minor sampling instants respectively.

Estimator II: Let us now consider the problem of estimating the nutrient concentrations from on-line and offline measurements. The relevant system equations are given as,

$$s_1(i+1) = -k_s(i)\ \tau\ \mu(i)\ x_1(i) + s_1(i) + Fs_{in} + w_s(i) \tag{7.31}$$

$$s_2(i+1) = s_1(i) \tag{7.32}$$

$$s_3(i+1) = s_2(i) \tag{7.33}$$

$$k_s(i+1) = k_s(i) + w_2(i) \tag{7.34}$$

Consider the observability of the augmented system S=[s_1 s_2 s_3 k_s] from off-line, delayed nutrient measurements. The discrete state transition matrix after linearization of the above system of equations can be written as,

$$\Phi = \begin{bmatrix} 1 & 0 & 0 & -\tau\mu(i)x_1(i) \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \tag{7.35}$$

The measurement vector and the measurement equation for this case can be written as,

$$z_{major\,JI}(i) = s_3(i) + \mathrm{v}_{major\,JI} \tag{7.36}$$

$$G_{major\,JI} = s_3(i) \tag{7.37}$$

Assuming that $G_{major,II}=\partial G_{major,II}/\partial S$, the structural observability matrix can be symbolically written as,

$$O = \left[G_{major\,JI}\ \ G_{major\,JI}\Phi\ \ G_{major\,JI}\Phi^2\ \ G_{major\,JI}\Phi^3\right]^T \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & -\tau\mu(i)x_1(i) \end{bmatrix} \tag{7.38}$$

Using the symbolic manipulation capabilities of Maple (1981), it can be verified that, as long as μ is non-zero, the above observability matrix does not have any structural deficiencies and is of full rank. Thus, in addition to the system states in the vector S, the parameter k_s can also be estimated at major sampling instants for a non-zero value of the specific growth rate. At the minor sampling instants, a similar structural observability analysis indicates that, because there is no assumed dependence of μ on the nutrient levels in equation (6), the latter are unobservable from CER measurements. Thus it is not possible to use the growth rate related measurements available at the minor sampling instants to update the state vector S. However, the estimates of the specific growth rate and the biomass generated at the minor sampling instants by Estimator I can be used to integrate the relevant state equations and yield estimates of the state S as proposed by Stephanopoulos and San(1984). In terms of the measurement and time update equations of the Kalman filter, we perform a time update or prediction using the most recent state estimates available at the current sampling instant. For estimator II, at the major sampling instant, we use the delayed nutrient measurement to perform a measurement update as given in equations (7.39-7.42) below and generate estimates of S. At the minor sampling instants, we simply set the time update to be equal to the measurement update. The estimator equations can be expressed as :

(i) Measurement update (Major sampling instant) :

$$K_{major,II} = M_{II}\, H_{major,II}(H_{major,II} M_{II} H^{T}_{major,II} + R_{z,major,II})^{-1} \tag{7.39}$$

$$\varepsilon_{major,II}(i) = z_{major,II} - G_{major,II} \tag{7.40}$$

$$\underline{\hat{S}}(i) = \underline{\bar{S}}(i) + K_{major,II}\varepsilon_{major,II}(i) \tag{7.41}$$

$$P_{II}(i) = M_{II}(i) - K_{major,II} H_{major,II} M_{II}(i) \tag{7.42}$$

(ii) Measurement update (Minor sampling instant) :

$$\underline{\hat{S}}(i) = \underline{\bar{S}}(i) \tag{7.43}$$

$$P_{II}(i) = M_{II}(i) \tag{7.44}$$

The time update at all sampling instants is performed, as in Estimator I, by an actual integration of the relevant system equations. These yield predictions of the state S for the next sampling instant. The prediction of the covariance matrix M_{II} is obtained by an actual integration of the equation :

$$M_{II}(i+1) = F_S P_{II}(i) F_S^T + Q_{II} \qquad (7.45)$$

The overall block diagram of the estimation scheme is shown in Figure 7.1.

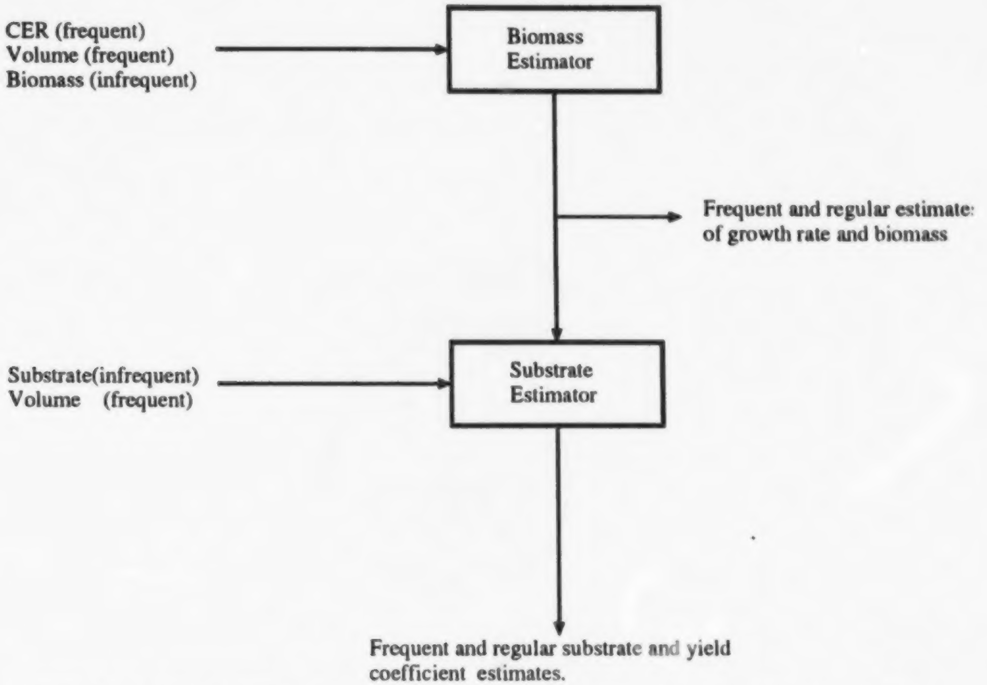

CER (frequent)
Volume (frequent)
Biomass (infrequent)
Biomass Estimator
Frequent and regular estimate of growth rate and biomass
Substrate(infrequent)
Volume (frequent)
Substrate Estimator
Frequent and regular substrate and yield coefficient estimates.

Figure 7.1 Block diagram for the biomass and substrate estimation algorithm.

The significant differences in the estimation scheme proposed here over that proposed by Stephanopoulos and San (1984) are as follows. For estimator I, incorporation of the infrequently measured biomass measurement and use of the modified measurement equations (18) and (24) yields unbiased state and parameter estimation even in the presence of a changing maintenance coefficient. Such estimators are thus useful for a wider class of fermentations that involve a changing maintenance coefficient. The observation scheme of Stephanopoulos and San (1984) is applicable to a different class of fermentations where the maintenance coefficient can be assumed to be constant or negligible. In addition, in this work the infrequent and delayed measurement of the nutrient concentration is formally incorporated into the estimation scheme in estimator II, thus making the estimation scheme proposed here closed-loop based, albeit at the major sampling instants only. To track the changing substrate to biomass yield coefficient, Stephanopoulos and San (1984) propose an approach using elemental balances based on an assumed stoichiometry of the fermentation reactions which need to be re-established for every new fermentation. The approach proposed here uses the delayed and infrequent nutrient measurement to update any change in the yield coefficient. It is thus able to accommodate shifts in stoichiometry with time in a fed-batch fermentation as well. Although the yield coefficient updating is relatively slower in the approach proposed here, no *a priori* knowledge of the fermentation stoichiometry is required. A few issues however need to carefully considered from an implementation viewpoint. In the industry, the biomass concentrations are usually measured in terms of the packed cell volume (PCV), which is a relatively unreliable measurement when there are suspended solids in the fermentation broth. However, recent work (Kennedy *et al.*(1992) in the development of a light scattering technique offers a promising solution to the above problem.

● **Remark** : In the above approach, it was necessary to separate the biomass and substrate estimation tasks because of observability problems. Such a classification of the overall system state into observable and unobservable subsystems has also been done by Schuler and Suzhen (1985) for a polymerization reactor. Due to unobservability of a few system states, the temperature, monomer and initiator concentrations are estimated in the first subsystem. These estimates act as inputs to a second subsystem in which the chain length distribution and its characteristic parameters are estimated.

7.4 Non-linear feedback control

Since the adaptive estimator described above generates estimates of the nutrient concentrations at a more frequent rate, a feedback control law based on these estimates can be used to obtain good regulation/tracking of the nutrient profiles by manipulating the nutrient addition rates. The dynamics of the nutrient concentration described in equation (7.2) is nonlinear (bilinear) and therefore a simple nonlinear control law based on feedback linearization (Slotine and Li(1991), Bastin and Dochain (1990), Chen *et* *al.*(1995)) has been used to design the control law. Let $s_{ref}(t)$ be the reference nutrient trajectory to be tracked and let the desired dynamics of the closed loop system be :

$$\frac{ds}{dt} = \eta_1(s_{ref}-s) + \eta_2\int(s_{ref}-s)dt \tag{7.46}$$

Since the right hand side of equation (7.2) has relative degree 1, we can equate it to the right hand side of equation (7.46) to obtain the non-linear control law,

$$F = \frac{V}{(s_{in}-s)}\left[-k_s\mu x+\eta_1(s_{ref}-s)+\eta_2\int(s_{ref}-s)dt\right] \tag{7.47}$$

7.5 Estimation Results

The proposed software sensor was analyzed using simulations as well as data from an industrial, secondary metabolite producing fed-batch fermentation. For the simulation study, a fed-batch antibiotic producing fermentation known to exhibit significant maintenance activity and endogenous metabolism was considered for verification.

7.5.1 Simulation Study

For numerical simulation, the system was modelled using dynamic balances along with empirical growth models for cell growth and product formation(Bajpai and Reuss(1980), Tsobanakis *et al.*(1991)) and dynamic gas balances for the gas phase (Cardello and San (1988)). The detailed equations are presented in Appendix A. Since the gas phase dynamics are relatively rapid as compared to the dynamics of the broth, the above system of equations is stiff. Therefore, a stiff equation solver LSODES (Hindmarsh,(1983)) available at the Lawrence Livermore Laboratories was used for the simulation. The system of dynamic balance equations was solved for a time interval of 120 hours for a linearly changing value of the maintenance coefficient m_c between 1.1 to 1.6 mmol CO_2/h-1. The initial conditions used in the simulation are also presented in Appendix A. The profiles of various culture states thus generated by simulation were then sampled according to the following multirate sampling scheme. Measurements of the primary process variables viz. biomass and substrate were assumed to arrive every 6 hours or 60 sampling instants of fermentation. Measurements of the CO_2 content in the exit gas and the broth volume were assumed to arrive every 6 minutes or every sampling interval. A delay of 2 minor sampling instants (12 minutes) was assumed for the primary measurements. White gaussian noise with zero mean was added to the measurements to simulate noisy measurements with a relatively smaller signal to noise ratio. The objective of the estimator was to estimate the nutrient (substrate), biomass concentrations and the system parameters on a frequent basis (i.e every 6 minutes) using the measurements arriving at multiple rates of sampling as described above.

Base case Estimation: Figure 7.2 shows the state estimates generated from the adaptive estimator. It can be seen that excellent tracking of biomass and substrate profiles is achievable. Figure 7.3 shows the parameter estimates generated by the estimator. It can be seen that unbiased estimates of the parameters are obtained using the adaptive estimator. The choice of ratio of the process noise covariance to the measurement noise covariance matrices is very critical to the estimator performance. By appropriate specification of these covariance matrices, greater confidence or weighting can be given to the primary measurements relative to the state predictions at the major sampling instant. Accordingly, at the major sampling instants, corrections in the state estimates can be seen for the biomass profiles. These corrections are relatively small for the substrate estimates.

Effect of initial guess of the yield coefficient: In a typical fermentation involving complex nutrients, the yield coefficient is generally unknown. To analyze the estimator performance when the yield coefficient is poorly known, a 10 fold mismatch in the yield coefficient was presented to the estimator as an initial guess. Figures 7.4 and 7.5 show the adaptation of the substrate to biomass yield coefficient. An initial guess of k_s=9 was used while the actual value of the yield coefficient used in the simulation was 0.9. It can be seen that the algorithm updates the yield coefficient quite well. Since the yield coefficient is unobservable from measurements at the minor sampling instants, the updating is done only at the major sampling instant. The yield coefficient values remain constant at the minor sampling instants.

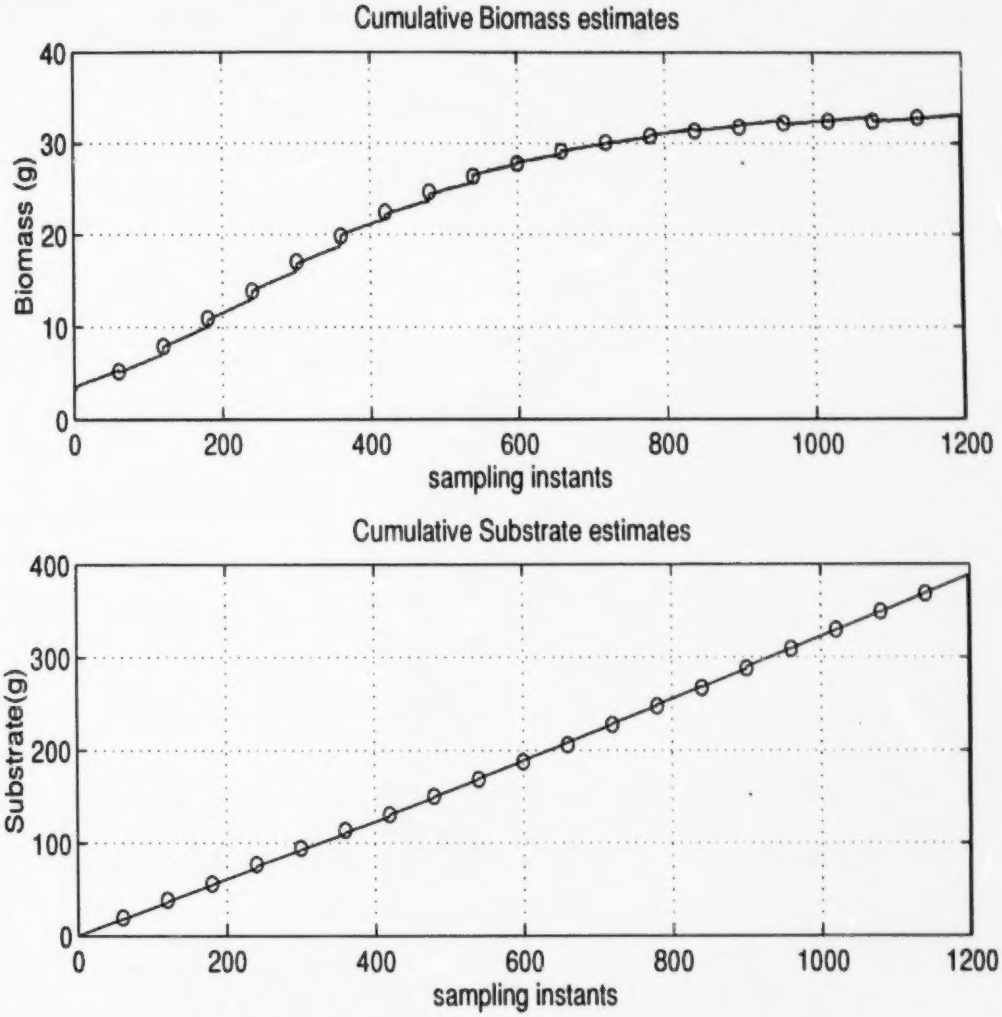


Figure 7.2 Base case estimation: The state estimates generated by the estimator (solid line) agree quite well with the values available from simulations(o).

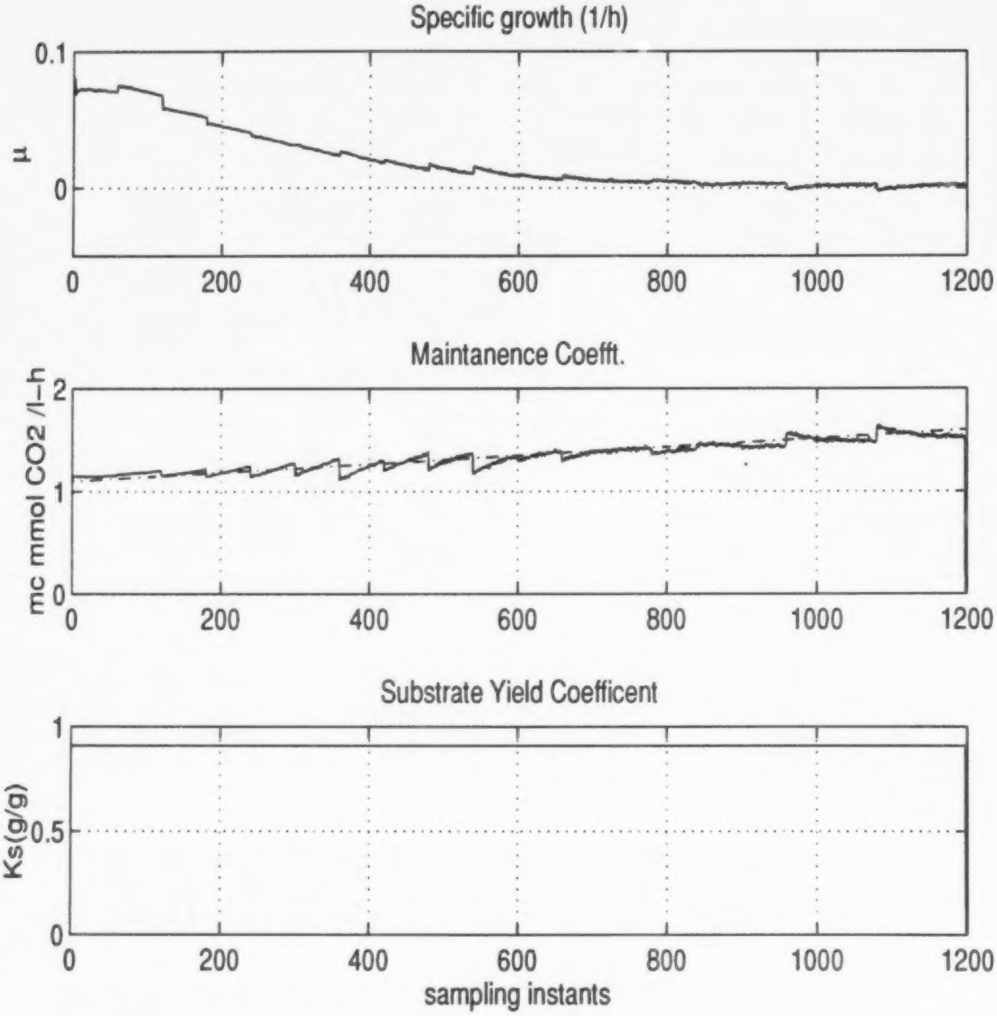


Figure 7.3 Base case estimation: The parameter profiles generated by the estimator (solid line) are quite close to the values specified in the simulations (.-)

Effect of measurement delay: Measurement delays generally have the effect of reducing overall system observability. For the biomass estimator (estimator I), the system observability can be enhanced by the addition of past inferential(secondary) measurements as shown in equations (18) and (24). However, the nutrient concentrations and the yield coefficient are unobservable from the secondary measurements. Thus, measurement delays in the nutrient concentrations can significantly affect the performance of estimator II. Figure 7.6 and 7.7 show the effect of an increase in the measurement delay from 2 sampling instants (12 minutes) to 5 sampling instants (30 minutes). It can be seen that the estimator performance deteriorates quite significantly because of the measurement delay. The updating of the yield coefficient is very small. The estimator performance for a high gain estimator is also seen in Figure 7.6 and 7.7 The filter gain relating the yield coefficient to the innovations in the nutrient concentrations is increased by specifying a higher covariance entry for the yield coefficient. This change does make the filter sensitive and capable of adapting to time varying dynamics. However, a high filter gain also makes the estimator increasingly sensitive to measurement noise.

Effect of a changing yield coefficient : In typical fermentations, due to metabolic shifts or varying culturing maintenance requirements, the yield coefficient may be slowly time varying. It would be desirable to track this change in the yield coefficient as this would generate accurate estimates of the rate of substrate depletion. An important point that must be taken into account here is that a changing yield coefficient can be tracked only so long as the specific growth rate is non-zero. If the specific growth rate takes on values close to zero, the depletion term $-k_s\mu x$ approaches zero. The estimator is then unable to detect a mismatch in the yield coefficient and will therefore not update it. To illustrate this and to study the estimator performance under such a scenario, simulation data was generated by varying the yield coefficient from 10 to 20. The dotted line in Figure 7.8 shows the substrate estimation profile for a changing yield coefficient during the 45 hours of fermentation. It can be seen that a changing yield coefficient gives biased estimates of the cumulative substrate. Drastic corrections to the state estimates via the measurement update

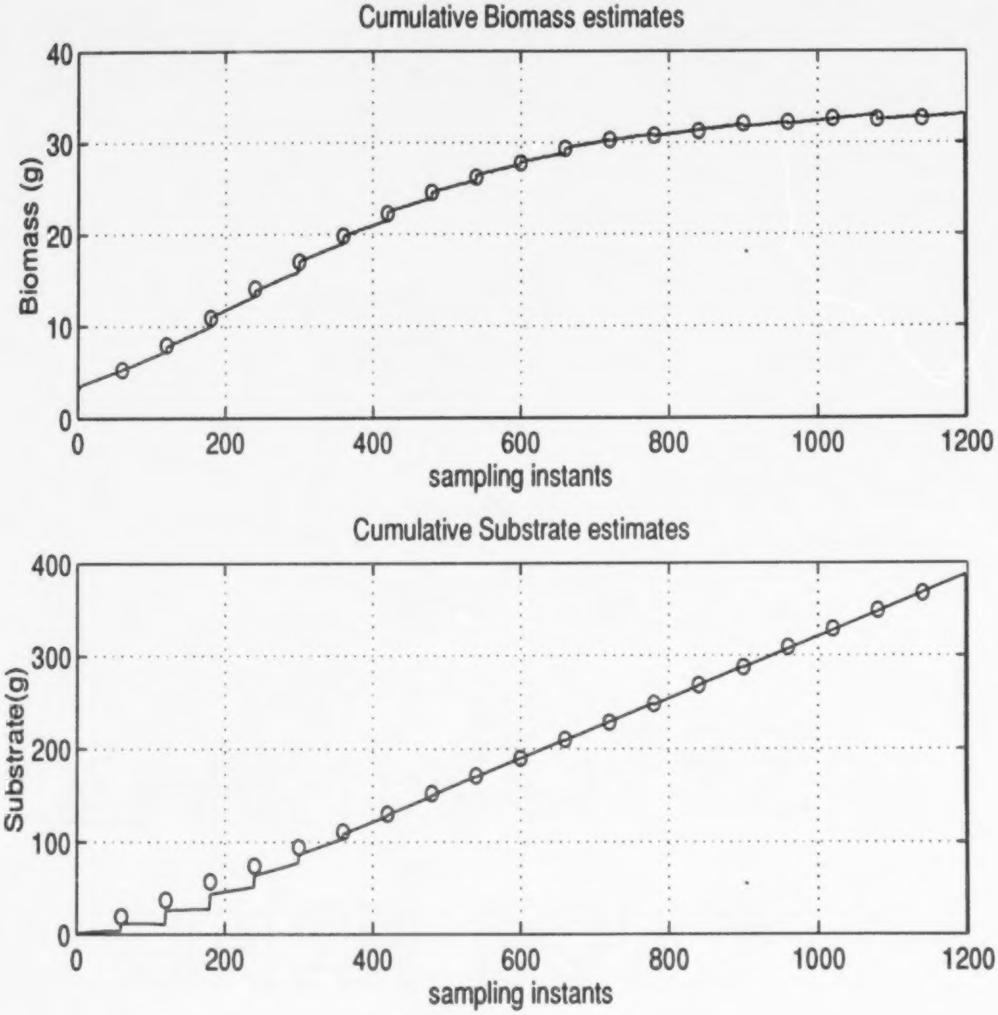


Figure 7.4 Effect of poor initial guess of the yield coefficient on the state estimates: The biomass estimates (solid line) compare quite well with the simulated values (o). However, in the initial instants, the substrate estimates are not good when there is significant mismatch in the yield coefficient.

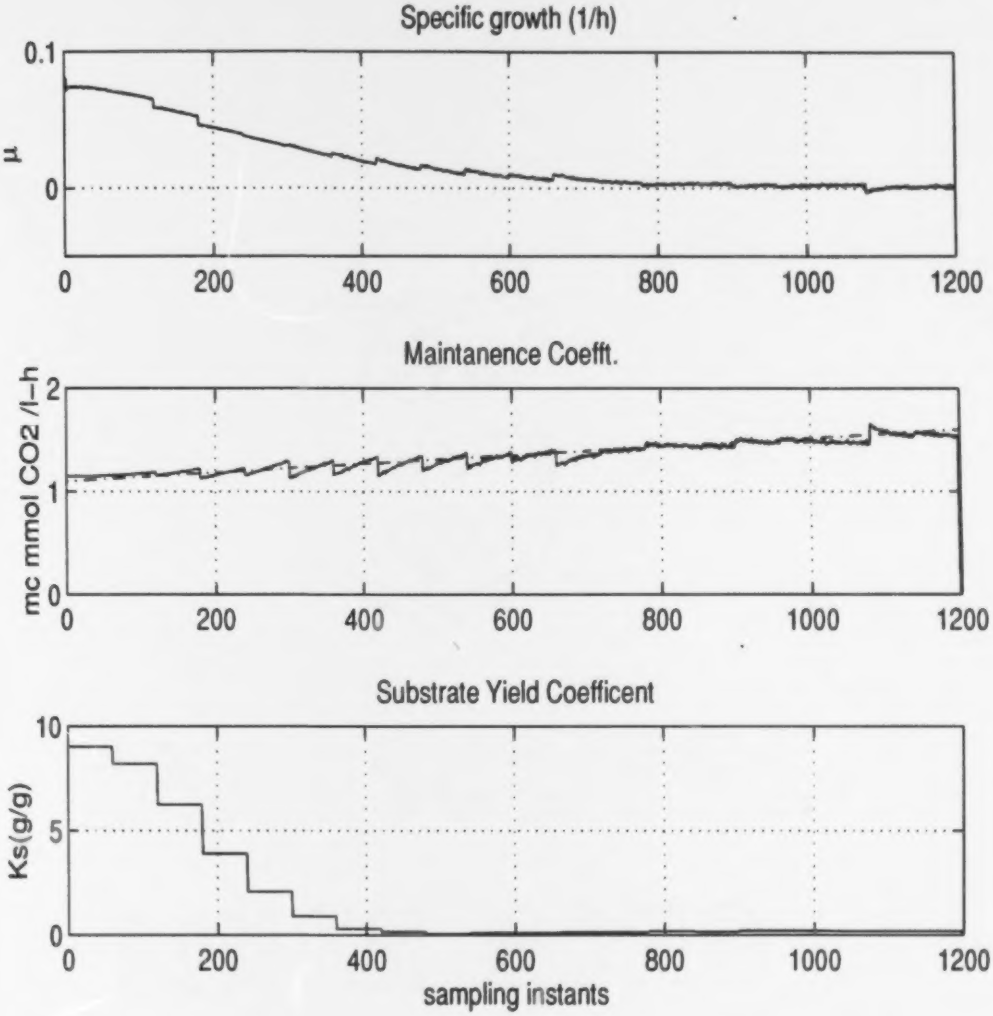


Figure 7.5 Estimation of the yield coefficient starting from a poor initial guess: The specific growth rate and the maintenance coefficient estimates (solid line) are relatively unaffected and agree with the values used in the simulations(-.). The yield coefficient is updated at the major sampling instants and converges quickly to the true value.

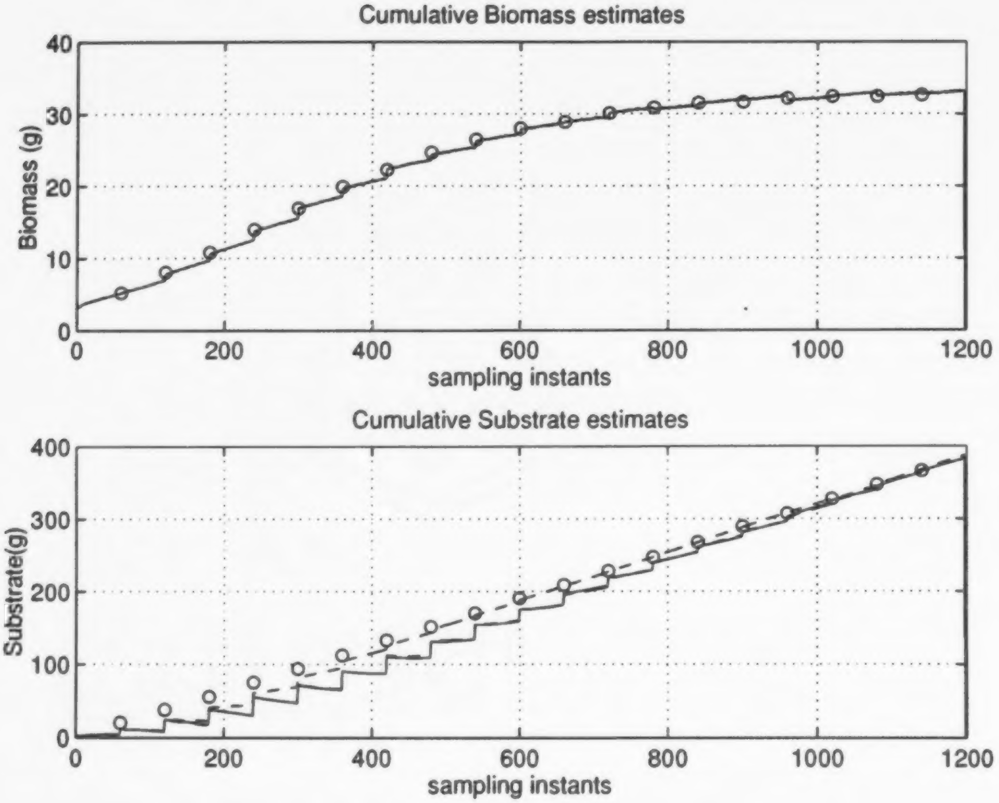


Figure 7.6 Effect of measurement delays in the primary measurements on the state estimates : For an increase in measurement delay from 12 minutes to 30 minutes in the nutrient measurements, the substrate estimates (solid line) differ significantly from the simulated values (o). When a high gain estimator is used, the substrate estimates (--) agree quite well with the simulations.

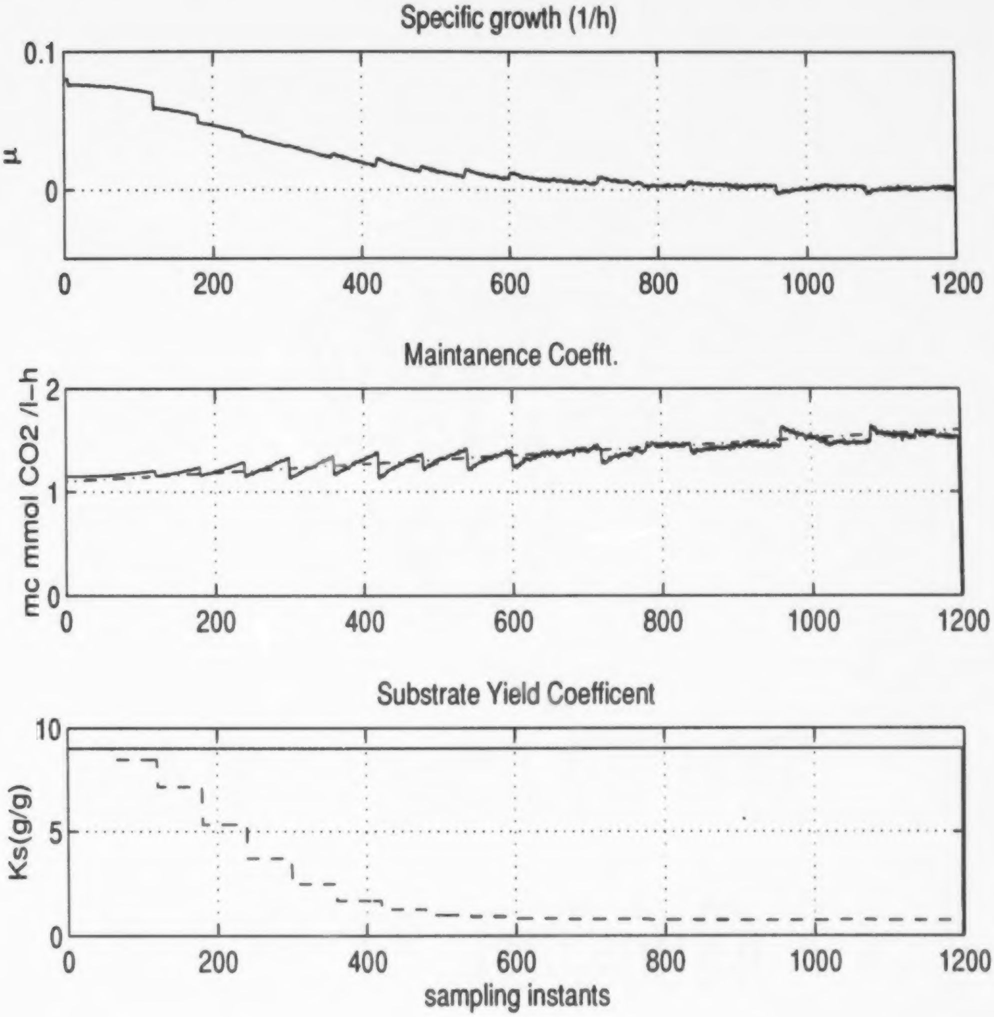


Figure 7.7 Effect of measurement delays in the primary measurements on the parameter tracking : The yield coefficient estimates (solid line) are not updated at all for the smaller gain estimator. For the higher gain estimator, the estimates (--) converge quickly to the true values.

equation of the Kalman filter is seen at the major sampling instants. By appropriate specification of the covariance term that corresponds to the yield coefficient variation in the overall process noise covariance matrix, the estimator was tuned to track the changing yield coefficient. The solid line in Figure 7.8 shows that good tracking of the cumulative substrate is achievable. Figure 7.9 shows the adaptation of the yield coefficient. The updating is done relatively slowly and is in fact very small after about 45 hours (450 sampling instants). This is to be expected because the value of the specific growth rate approaches zero after about 450 sampling instants. The observability matrix defined in equation (38) is rank deficient under such conditions and the system is only partially observable. More specifically, the effect of a changing yield coefficient is not seen in the substrate profiles. Figure 7.10 shows the cumulative substrate estimates for the overall run. It is seen the estimation for the fermentation duration after 45 hours is good. It is unaffected by the changing yield coefficient.

7.5.2 Verification using industrial data

The adaptive estimator was validated using data from an industrial, fed-batch, antibiotic fermentation reactor involving a streptomyces specie. After 30 hours of batch growth, a linear nutrient addition rate is followed during the fed-batch phase of the fermentation. The measurements that were available from the fed-batch fermentation and the frequency of availability are presented in Table 1. The actual values of the process variables and the results are presented on a normalized scale in Figure 7.11.

As a first step, the packed cell volume(α) measurements were converted to biomass concentrations(X) using the equation,

$$X = \alpha \rho_c f \tag{7.48}$$

where ρ_c is the bulk density and f is the ratio of the dry cell weight to the wet cell weight. The value of f was obtained by performing simple gravimetric analysis in the region of interest. Both ρ_c and f were approximately constant throughout the fermentation. For the estimator, a measurement delay of 2 minor sampling instant (30 minutes) was used in the model. A value of 1.1 mmol CO_2/h-g biomass was assumed for

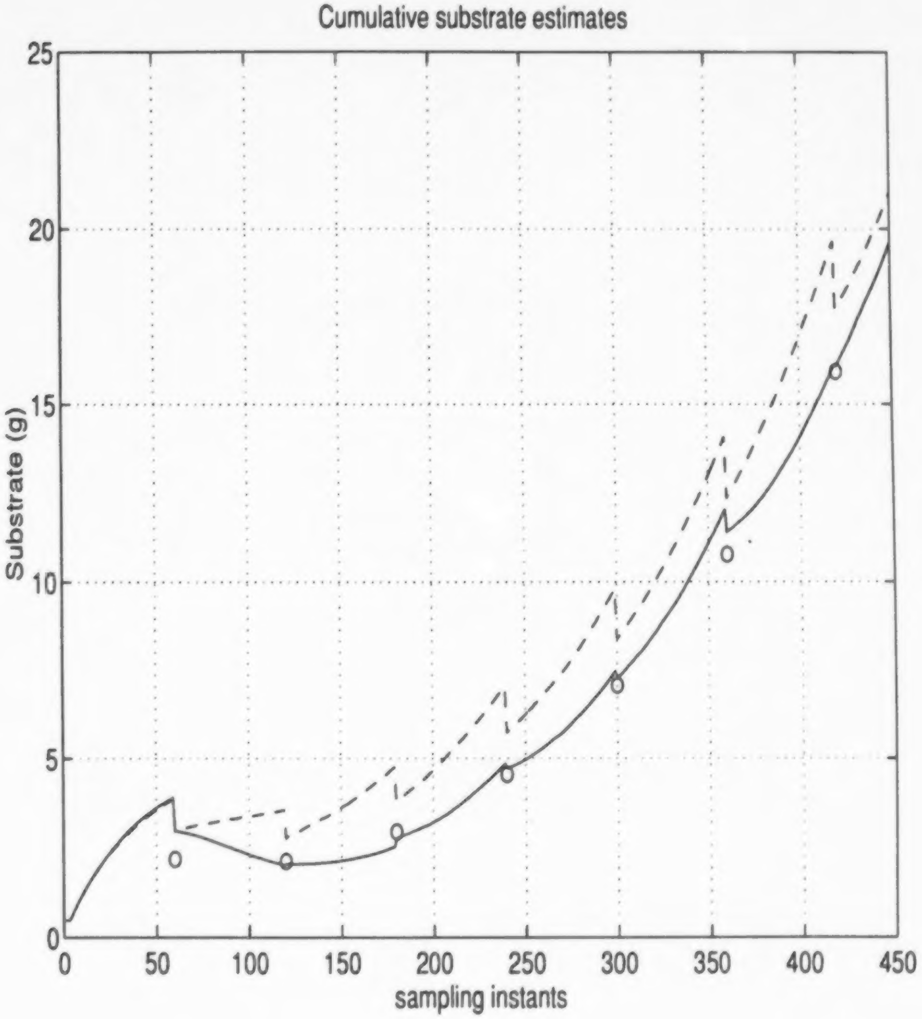


Figure 7.8 Effect of a changing yield coefficient on the substrate concentration estimates. Biased estimates and drastic corrections at the major sampling instants are seen (dotted line) in the substrate estimates. The solid line shows the substrate estimates when the yield coefficient is adapted to the time varying dynamics.

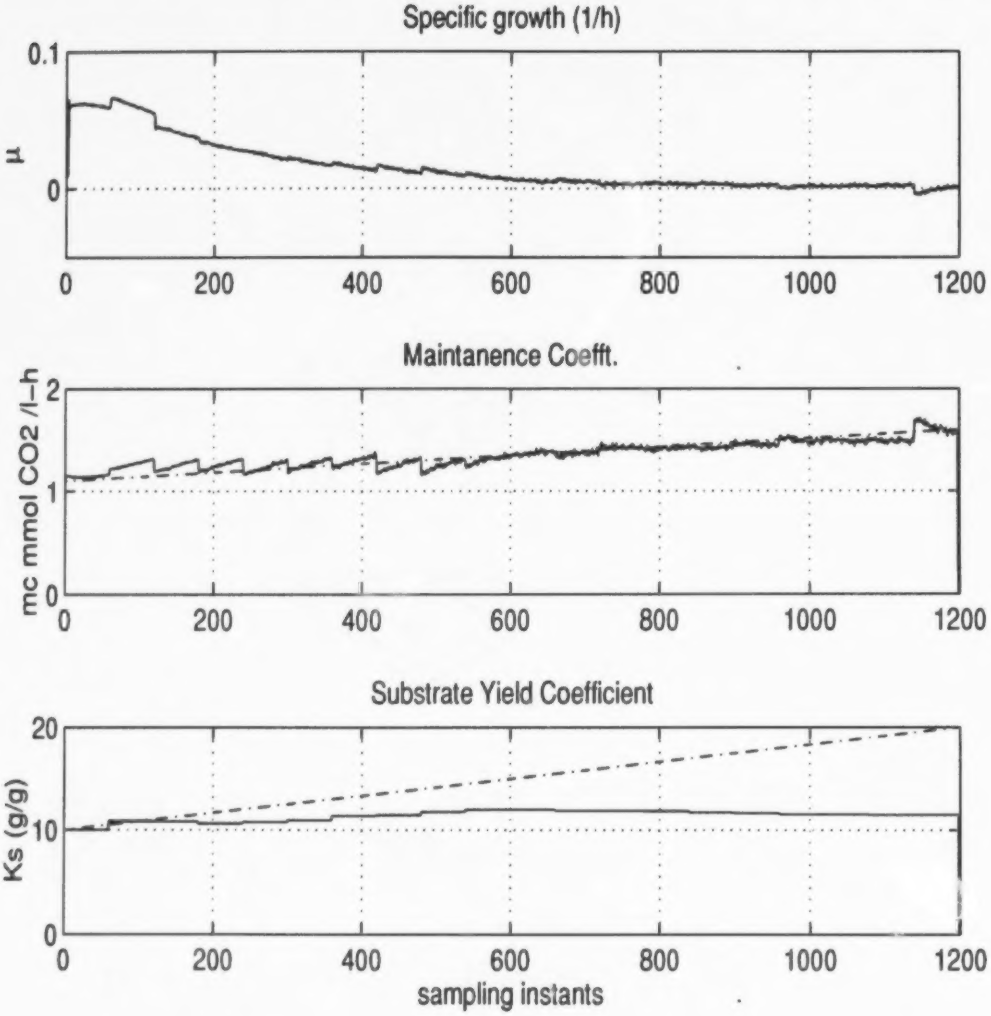


Figure 7.9 Adaptation of the yield coefficient. The adaptation is performed only when the specific growth rate is non-zero. As the specific growth rate approaches zero, the yield coefficient is unobservable from the substrate measurements and cannot be updated at the major instants.

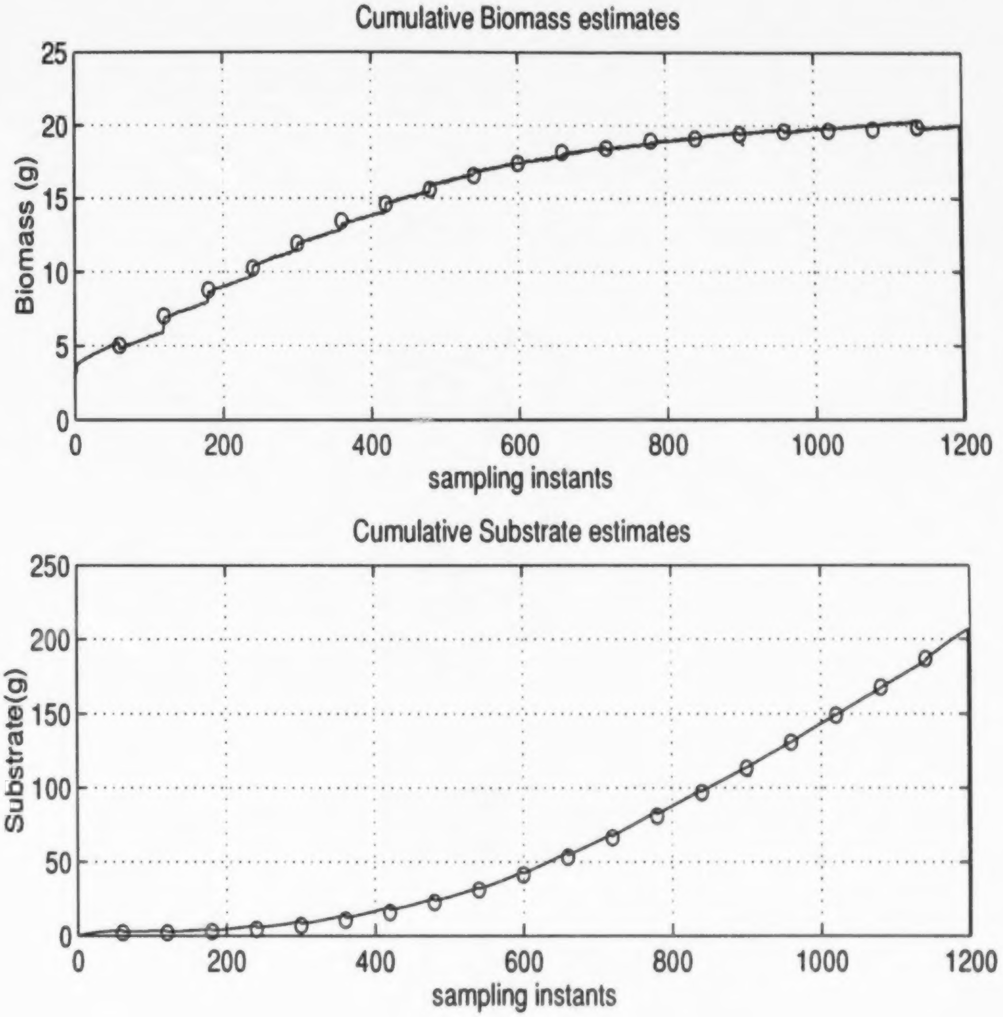


Figure 7.10 Profiles of the state estimates for a changing yield coefficient over the entire fermentation run.

the yield coefficient k_1 (Pirt and Righelato, (1967)). The estimator was initialized with appropriate values of the parameters. The initial conditions for the fermentation states were assumed to be the same as that measured in the fermentation. Prefiltering of the CER measurements was performed using a Butterworth filter to remove high frequency sensor noise. Outliers and gross errors in the measured PCV values were detected and removed using a statistical prefilter that was constructed using the datasets from each fermentation run. The PCV profiles were interpolated to get "measurements" every 6 hours. In the absence of information regarding the time evolution of the parameters, a trial and error based tuning procedure was used to tune the process noise covariance matrix relative to the measurement noise covariance matrix. Accordingly, the process noise and measurement noise covariance matrices were chosen as specified in Table 1.

Figure 7.11 and 7.12 show the performance of the adaptive estimator for one of the representative runs of the fermentation. It can be seen that excellent tracking of the biomass and the nutrient concentrations can be obtained. The real potential of this strategy is better seen when the estimator operates in an on-line manner. In such a scenario, the primary measurements are not available at all times and the estimator has to predict the biomass and substrate values at sampling instants between the primary measurements. It can be seen from Figure 7.11 that the estimates of the biomass and the nutrient concentrations at the minor sampling instants are quite good. The adaptive estimator thus provides accurate inferential estimates of the states at the minor sampling instants and therefore has good potential for use in an on-line control scheme for regulation/tracking of nutrient levels.

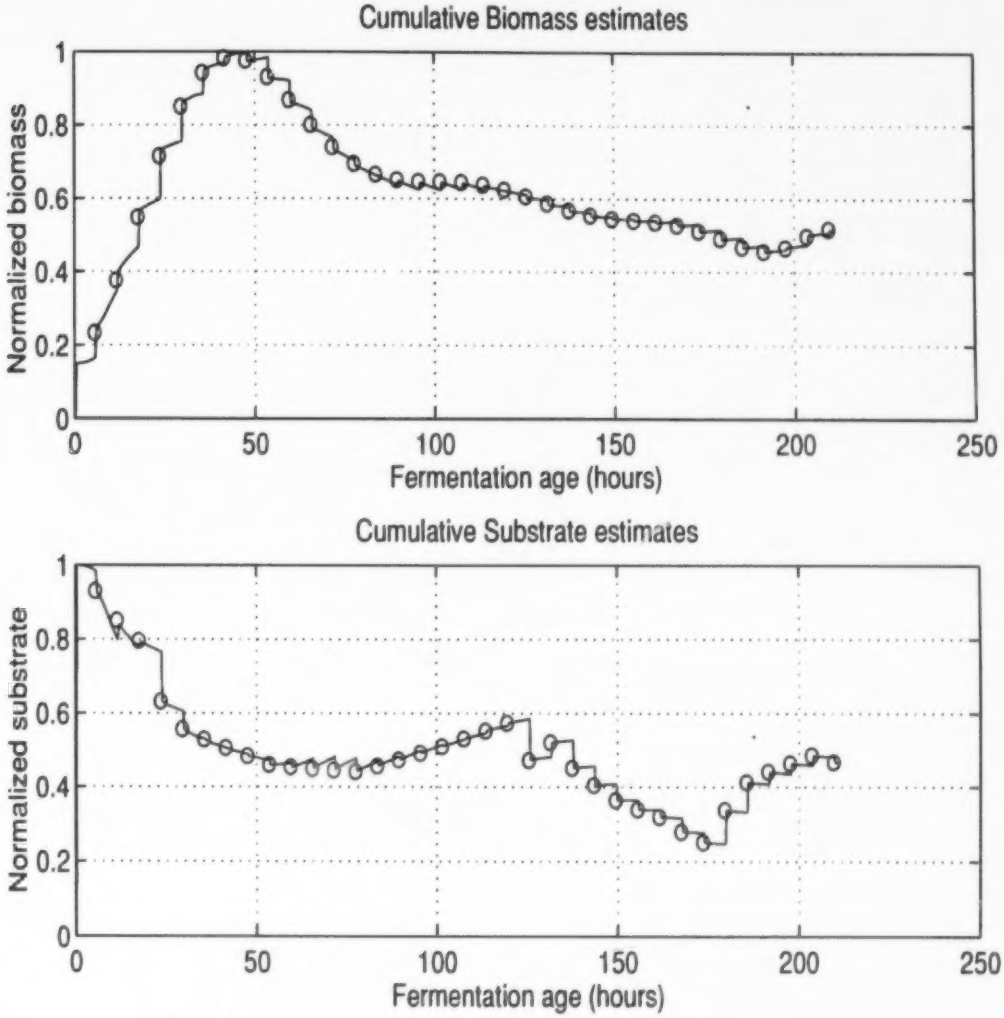


Figure 7.11 Estimator validation using industrial data: State estimates (solid line) agree quite well with the measured values (o). The estimates in between major sampling instant are quite consistent.

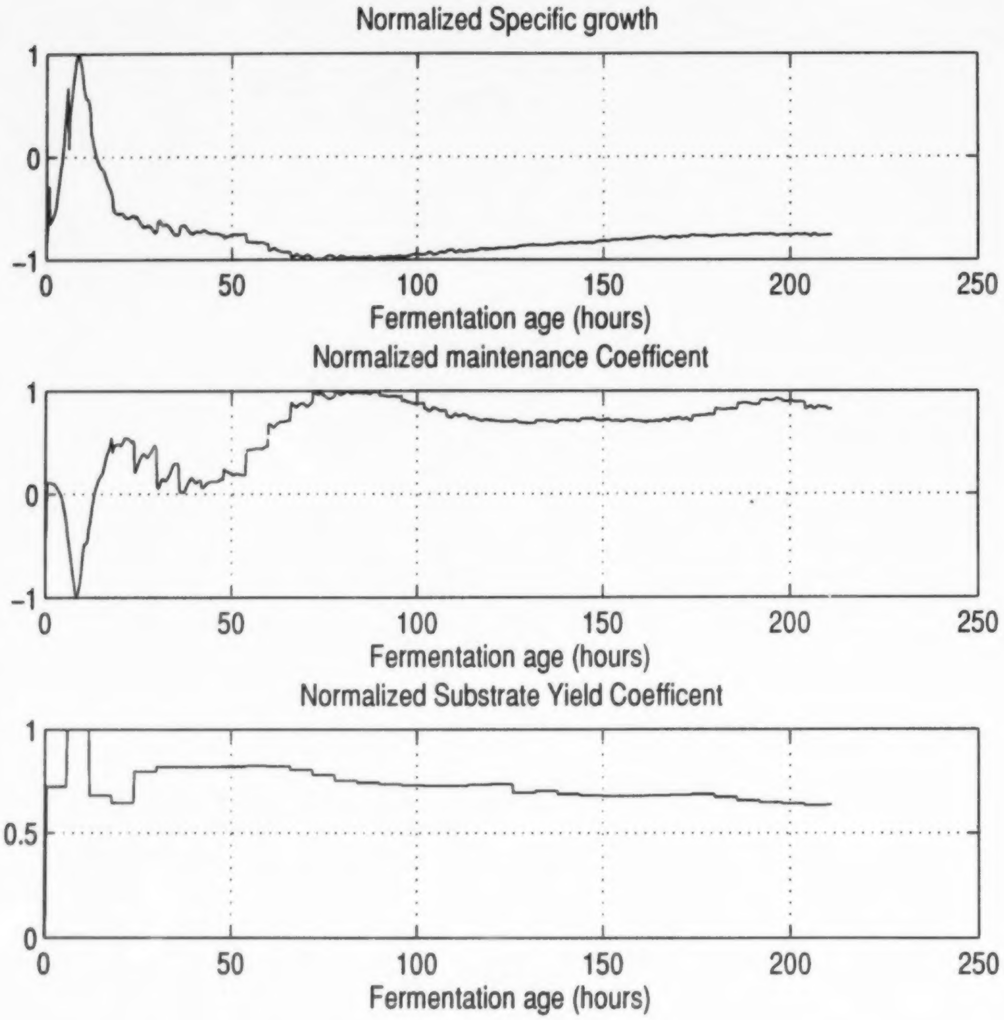


Figure 7.12 Estimator validation using industrial data: Parameter estimates for the industrial run.

7.6 Control Performance

The performance of the non-linear control law defined in equation (7.47) was verified using simulations involving the fed-batch fermentation described above. The fortan code, for simulating the system dynamics (Appendix-A) was interfaced to MATLAB® using the Matlab external interface engine. The estimator was run under MATLAB® and received "measurements" from the external interface. The appropriate value of the nutrient feeding rate was calculated and sent through the interface to be implemented on the "process". For verification, a simple first order reference profile was specified for the nutrient concentration trajectory. The algorithm was evaluated for its ability to track the reference profiles by manipulating the nutrient feeding rate even in the presence of significant mismatch in the yield coefficient. Figure 7.13 shows the nutrient concentration profile and the nutrient feeding rate profile for the above objective with values of η_1=0.6 and η_2=0. It can be seen that excellent tracking of the nutrient concentrations is achievable. Figure 7.14 shows the effect of a mismatch in the yield coefficient. The control actions in the nutrient feeding rate is very erratic when there is significant mismatch in the yield coefficient. However, as the estimated value of the yield coefficient approaches the true value, the nutrient additions become less erratic and good setpoint tracking is observed.

7.7 Conclusions

Problems associated with estimation and control of nutrient levels in a fed-batch fermentation are discussed and addressed in this paper. An adaptive multirate estimator has been proposed to formally incorporate delayed and infrequent, off-line measurements of the primary process variables, such as the biomass and substrate concentrations, into traditional inferential estimation strategies. It has been shown that these off-line measurements enhance estimator performance by providing 'closed loop' estimates of the states and parameters. The adaptive estimator has been extensively validated using simulations and industrial data from a secondary metabolite fermentation. The estimator has been used in conjunction with a nonlinear control law. The control law has been

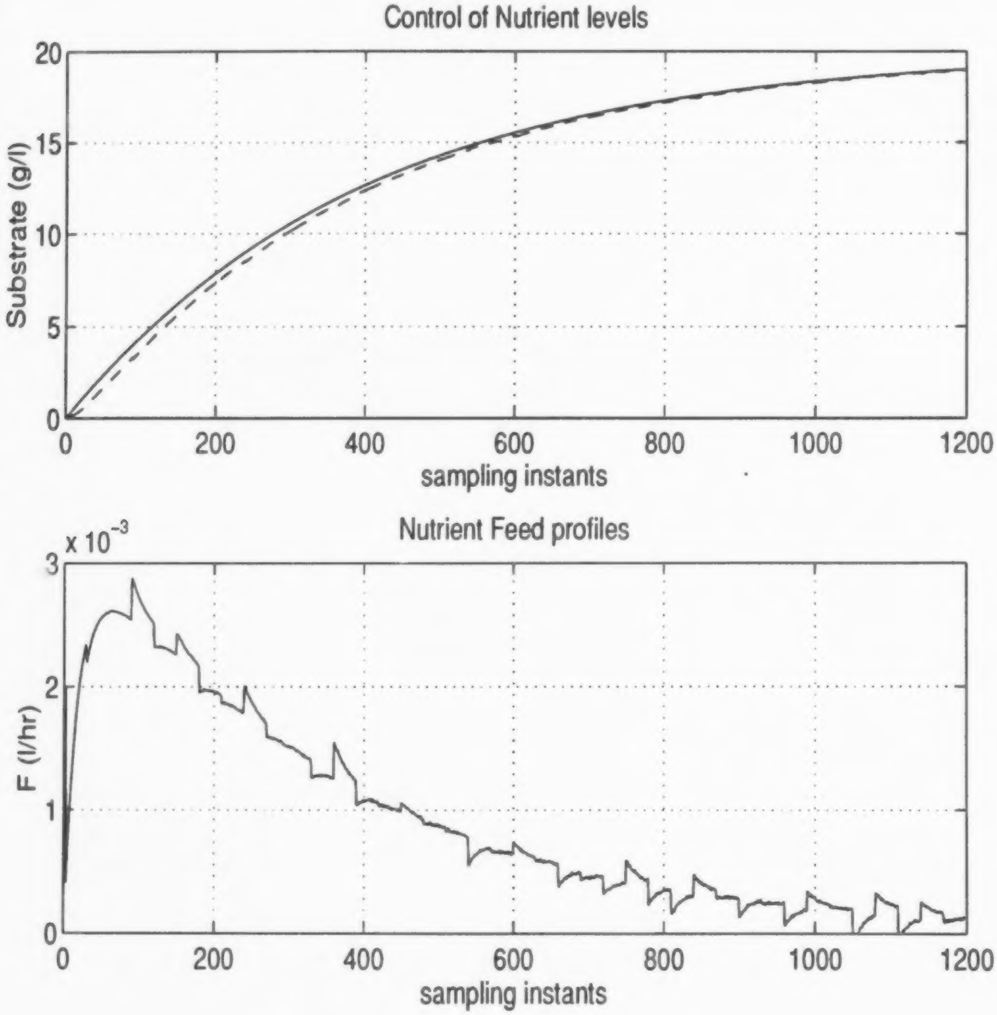


Figure 7.13 Nonlinear control of the nutrient levels. The nutrient concentrations (solid line) tracks the reference trajectory (--) quite well.

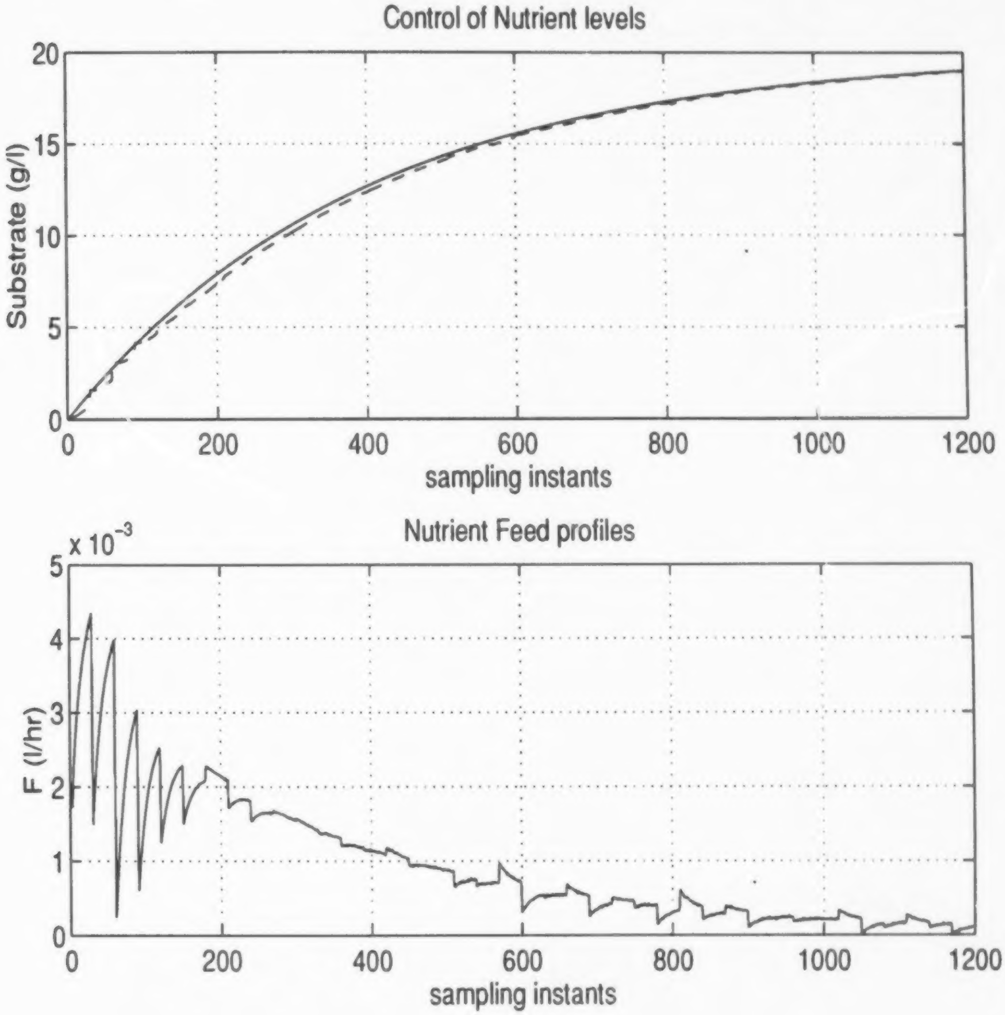


Figure 7.14 Nonlinear control of the nutrient levels in the presence of yield coefficient mismatch. The feeding rate is a little erratic when the mismatch is significant. As the correct value of the yield coefficient is identified, the feeding rate profiles become more regular and consistent.

successfully verified using simulations involving a fed-batch fermentation and has been shown to give good performance even in the presence of significant mismatch in the yield coefficients.

7.8 References

Bajpai, R.K and Reuss, M., " A mechanistic model for Penicillin Production", J. Chemical Technology and Biotechnology, **30**, p 332,(1980).

Bastin, G. and Dochain, D.,"Online estimation and adaptive control of Bioreactors", Elsevier Science Publishing Co. Inc, New York, USA (1990).

Cardello, R.J and San, K.Y., "The design of controllers for batch bioreactors", Biotechnology and Bioengineering, **32,** p 519, (1988).

Chen, L., Bastin, G. and Van Breusegum, V., " A case study of adaptive nonlinear regulation of Fed-batch Biological reactors.", Automatica, **31**(1), p 55,(1995).

Franklin, G.F., Powell, J.D. and Workman, M.L., "Digital control of dynamic systems", Addison-Wesley Publishing Inc., Reading, Massachussets, USA (1990).

Glasson, D.P., " Research in Multirate estimation and control", Analytic Sciences Corporation, Six Jacob Way,Reading, Massachussets, USA (1980).

Glasson, D.P., "Development and Applications of Multirate Digital Control ", IEEE Control Systems Magazine, **3**(4), p 2,(1983).

Gudi, R.D., Shah, S.L., " The Role of Adaptive Kalman filter as a software sensor and its application to a bioreactor", Proceedings of the 12th IFAC World Congress, **8,** p 221, Sydney, Australia (1993a) .

Gudi, R.D., Gray, M.R. and Shah, S.L., "Multirate estimation and monitoring of Process Variables in a bioreactor", Proceedings of the 2nd IEEE Conference on Control Applications, **1**, p 487-492, (1993b).

Gudi, R.D., Shah, S.L. and Gray,M.R., "Multirate state and Parameter estimation in an Antibiotic fermentation with delayed measurements", Biotechnology and Bioengineering, **44**,1271,(1994).

Gudi, R.D., Shah, S.L. and Gray, M.R., "Adaptive multirate state and parameter estimation strategies with application to a bioreactor", Accepted for publication in the AIChE Journal (1995).

Hindmarsh, A.C., "Odepack : A systematized collection of ODE solvers", *In* "Scientific Computing", (Ed) Stepleman, R.S., p55-64, North Holland Publishing Co., Amsterdam, (1983).

Kennedy, M.J., Thakur, M.S., and Wang, D.I.C and Stephanopoulos, G.N., "Estimation of cell concentrations in the presence of suspended solids - A light scattering technique", Biotechnology and Bioengineering, **40**(8), p 875, (1992).

Lawrence Livermore National Laboratories, Livermore, CA 94550,(1987).

Maple, Waterloo Maple Software, 450 Phillips St., Waterloo, Ontario, Canada N2L 5J2, (1981).

Park, S. and Ramirez, W.F., "Optimal regulatory control of bioreactor nutrient concentration incorporating system identification", Chemical Engineering Science, **45**(12), p 3467, (1990).

Pirt, S.J. and Righelato, R.C.," Effect of growth on the synthesis of penicillin by *Penicillium chrysogenum* in batch and chemostat cultures", Applied Microbiology, **15**, 1289,(1967).

Ramirez, W.F., "Optimal state and parameter Identification : an application to batch fermentation", Chemical Engineering Science, **42**(11), p 2749, (1987).

Ray, W.H., "Advanced Process Control", McGraw-Hill Book Company, New York, USA (1981).

Schuler, H and Suzhen, Z., "Chain length distribution in a polymerization reactor", Chemical Engineering Science, **40** (10), p1891, (1985).

Slotine, J.E.and Li, W., "Applied Nonlinear control", Prentice Hall, (1991).

Stephanopoulos, G. and San, K.Y.," Studies on on-line bioreactor identification", Biotechnology and Bioengineering, **26**, p1176,(1984).

Stone, K.M., Thornhill, N.F., Roche, F.W and Fish, N.M," A method of using off-line measurements in an on-line estimator of biomass concentration for a penicillin fermentation and its effects on the quality of the estimates", 2nd IFAC symposium on Modelling and Control of Biotechnological processes and 5th International Conference on control applications to Fermentation Technology, Keystone, Colorado,(1992).

Tham, M.T., Montague, C.A., Morris, A.J. and Lant, P.A., " Soft sensors for process estimation and inferential control", J. Proc. Control, **1**, p3, (1991).

Tsobanakis, P., Lee, S.H., Phillips, J.A. and Georgakis, C., "Issues in the control and optimization of fed-batch bioreactors by tendency models", AIChE National meeting, Nov 14-15,1990.

TABLE 7.1

Measurement frequencies of the process variables for the industrial fermentation

Measurement	Frequency
% Packed Cell Volume	Infrequently.
Nutrient concentrations	Every 6 hours
Broth Volume measurements	Every 15 minutes
CER measurements	Every 15 minutes
Feed rates	Every 15 minutes

Process noise and Measurement noise covariance matrices for the Industrial fermentation

$$Q_I = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 2 \end{bmatrix}; \; Q_{II} = \begin{bmatrix} 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 50 \end{bmatrix}$$

$$R_{z,major,I} = \begin{bmatrix} 0.0011 & 0 & 0 & 0 \\ 0 & 1.2 & 0 & 0 \\ 0 & 0 & 1.2 & 0 \\ 0 & 0 & 0 & 1.2 \end{bmatrix}; \; R_{x,major,II} = [0.0051]$$

$$R_{z,minor,II} = 1.2 * \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

7.9 Nomenclature

Roman

k_s	substrate over biomass yield coefficient (g/g).
m_c	maintenance coefficient (mmol /l-hr).
s	substrate concentrations (g/l).
t	time variable for integration.
w	noise process with unspecified probability densities.
x	biomass concentrations.
z	vector of process measurements.
F	nutrient feeding rate.
F_X,F_S	Jacobian obtained after linearization of biomass and substrate subsystems
G	measurement equation expressed as a function of state variables.
M	*a priori* (before measurement) estimate of the covariance of estimation errors.
O	symbolic observability matrix.
P	*a posteriori* (after measurement) estimate of the covariance of estimation errors.
Q	process noise covariance matrix.
R	measurement noise covariance matrix.
S	superstate formed by augmenting substrate concentrations and the yield coefficient.
V	Volume of the broth.
X	superstate formed by augmenting biomass concentrations and the yield coefficient.

Greek

ε	innovations vector obtained in the Kalman filter equations.
η_1	controller tuning constant in equation (46).
η_2	controller tuning constant in equation (46).

Chapter 7: Multirate estimation and Control of Nutrient Levels.

μ	specific growth rate.
ν	measurement noise vector.
τ	sampling time for discretization.

subscripts

in	related to the process inputs.
x	related to the biomass concentrations.
s	related to the substrate concentrations.
major	pertaining to the major sampling instant.
minor	pertaining to the minor sampling instant.
I	used in the first (biomass) estimator.
II	used in the second (substrate) estimator.
ref	reference (*a priori* established) trajectory.
z	pertaining to the measurement.

Chapter 8

Statistical Monitoring of Fed-batch fermentations[1].

The recent shift in chemical process industries towards the production of high-quality, low volume specialty products has emphasized the need for monitoring of batch and fedbatch processes. Reproducibility of plant operations is difficult to achieve due to the absence of reliable process sensors, changes in feed conditions, process disturbances and the discontinous nature of the process itself. The main motivation of this chapter is the presentation of a monitoring algorithm that is based on the use of multivariate statistical tools. Unlike traditional first principal models, the monitoring algorithm does not need a mechanistic model and makes use of all available on-line and off-line measurements as well as *a priori* process information to detect and diagnose faults and to make final quality predictions. It achieves data compression in that fewer variables, in a suitably transformed space, need to be monitored. The monitoring algorithm is evaluated on a fed-batch antibiotic producing fermentation and is found to give quick and early detection of faults and good predictions of the final antibiotic titre.

---

[1] The work presented in this chapter has been carried out collaboratively with S. Lakshminarayanan in an on-going Ph.D research project. A version of this chapter has been accepted for presentation at the AIChE Annual meeting 1995 as : S. Lakshminarayanan, Ravindra D. Gudi, Sirish L. Shah and K. Nandakumar, "On-line Monitoring of a Fed-batch Fermentor using Multirate-Multiblock- Multiway Projection to Latent Structures".

8.1 Introduction

This article focuses on statistical monitoring of fermentation bioprocesses. In particular, it discusses the development of an online strategy to detect and diagnose, faults and deviations from prescribed plant behaviour using multivariate statistical tools. Such strategies also have useful applications in traditional chemical processes such as batch distillation and batch/semibatch polymerization.

Fermentation processes are excellent examples of very high value, low volume and demand specific specialty products that follow a proprietary, *a priori* established manufacturing recipe. The manufacturing costs are significantly high. The process is fraught with risks of contamination by foreign microorganisms and lower product yields due to suboptimal operation or deviations from prescribed behaviour due to disturbances. There is, therefore, a strong incentive to develop monitoring and fault diagnosis strategies for bioprocesses. An early detection of faults can help in taking corrective action, when possible, to alleviate the fault or to shut down the batch to prevent wastage of expensive feed material.

Bioprocesses in general and fed batch/batch fermentations in particular pose significant challenges from a monitoring viewpoint. Most importantly, the requirement of sterility or an aseptic environment in the reactor and a lack of adequate on-line sensors to measure the primary process variables severely restricts the frequency of sampling of the broth on a regular basis. Only a few infrequent measurements of the primary culture states such as the biomass and the substrate concentrations are available. This restriction influences the monitoring strategy that can be used. Since other secondary process variables are measurable quite frequently in a non-intrusive way, inferential estimation strategies need to be used. An inferential estimation strategy infers the primary process variables from other secondary measurements. In a bioprocess context, there are several such measurements like the carbon dioxide evolution rate (CER), the oxygen uptake rate (OUR), the acid/base addition rates and the pH. The second important limitation for monitoring in a bioprocess arises from a lack of detailed process knowledge about the fermentation. Due to the several intermediate reactions that occur to form the final product of interest such as the antibiotics or other intermediates, a detailed mechanistic process model is impossible to develop. Thus *model based* fault diagnosis strategies are difficult to implement in a bioprocess.

In the literature, the problem of monitoring batch processes has been tackled broadly in three ways (Nomikos and MacGregor, (1994a)). The first approach is based on using several state estimators in parallel, each based on models that characterize different modes of operation viz. the normal operation mode and different fault/failure modes (King (1986),(1992)). Although off-line measurements can be incorporated into a state estimation framework (Gudi *et al.*,(1995)), state estimators require regular measurements of the primary process variables. Such measurements are usually not available for bioprocesses. In an inferential setup, state estimators need a fairly accurate process model; a requirement that is again not met in a bioprocess context. To alleviate the convergence problems that usually result, MacGregor *et al.* (1986) propose the use of stochastic integrator states. Such state estimators need to be carefully tuned to achieve accurate reconstruction of the states. In general, state estimators need a careful choice of measurements that can be used in conjunction with the process model in that the system model and the measurement must form (mathematically) an observable pair. Thus only a subset of measurements that are commonly logged in regular plant operations can be used. State estimators function by constructing an accurate estimate of the plant states. This is useful only in the context of feedback regulation. For monitoring and fault diagnosis purposes, such an estimate is usually not required explicitly.

The second approach to the development of monitoring and fault diagnosis strategies is based on the use of expert systems and artificial intelligence based algorithms to construct a database of regular and faulty modes of plant operation (Venkatsubramanian and Chan (1989)). Any plant operation can then be classified as regular or faulty using a pattern classifier. The limitation of this approach lies in the construction of the database. Datasets that characterize the fault modes of plant operation are not easy to get from plant operations. Heuristics based expert systems that rely on capturing knowledge from plant personnel are also restrictive because they can take into account only as many fault occurrences and process variable interactions, that the plant personnel can envision.

The third approach to monitoring, which is also the focus of this paper, is based on the use of multivariate statistical tools to detect faults and abnormal plant operation. Plant data, in the form of measured outputs, is often highly correlated. In a fermentation process context, the measurements such as the CER, OUR are all correlated with one primary phenomenon that occurs in a bioprocess viz. the biomass growth. Multivariate statistics tools such as the principal component analysis (PCA) and projection to latent structures (PLS) can be used to analyze this highly correlated plant data and identify the lower dimension subspace of the process. In these *data-based* approaches, statistical models are developed using plant data collected during the normal plant operation. Plant data from a currently operating plant is compared with the template of "normal" conditions and diagnosed for process upsets and sensor failures (Kresta *et al*., 1991). These approaches are very appropriate for bioprocess monitoring because they obviate the need for understanding the process at a fundamental level and for using intrusive sensors that can contaminate the fermentation broth. All that is needed is data from normal plant runs that is routinely logged onto the database.

In this work an extension of the multiway PCA and PLS based algorithms, so as to include infrequent and off-line primary variable measurements, is proposed and applied to monitoring and on-line prediction of final product quality in a fed-batch antibiotic fermentation. In bioprocesses, data on primary variables such as biomass and substrate concentrations are available only every few hours due to lack of adequate online sensors. Elaborate offline laboratory procedures are required to arrive at these values. Multiway PCA and PLS based batch process monitoring algorithms that have been reported earlier (Nomikos and MacGregor, (1994b)) disregard the multirate nature typical of most systems. Often times, the intermediate and final quality variables such as the antibiotic concentrations are strongly correlated with the primary process variables. Incorporating off-line measurements of the primary variables during the model building step yields a statistical model that has better prediction capabilities. With its multirate focus, the proposed algorithm brings into direct focus this character of the measurement system. The multiblock feature of the proposed algorithm concerns itself with the various blocks that naturally emerge from partitioning the measurement system into blocks of initial conditions, primary measurements, secondary measurements and final quality data. The multiway feature is needed to consider several normal batches in the construction of the statistical model. Another novel feature of the proposed algorithm is the incorporation of *a priori* knowledge into the model building step. In a fermentation context, it is known that the underlying reactions are controlled by physiological mechanisms that are difficult to model explicitly. For example, the final antibiotic titre is strongly influenced by the initial growth rate of the microorganisms through the production of antibiotic precursors. Since such dependencies are difficult to model mechanistically, it is proposed to incorporate online estimates of the specific growth rate parameter into the prediction model to statistically model its influence on the final antibiotic yield. The resulting multirate-multiblock-multiway PLS (M3PLS) algorithm has been extensively verified using simulations and shown to give good prediction of the faults and final antibiotic concentrations when implemented on-line.

This chapter is organized as follows. Section 2 gives an overview of the measurement system for a fed-batch fermentation. Section 3 outlines the M3PLS algorithm. Section 4 presents the performance results obtained with the M3PLS algorithm. Section 5 presents the conclusions.

8.2 System description and Block diagram

The system considered in this work is a fed-batch, antibiotic producing fermentation. The model equations that describe the system dynamics are presented in Appendix A. Figure 8.1 shows the measurement system commonly associated with the fed-batch fermentation. The primary process variables or culture states are the biomass, substrate and antibiotic concentrations. The final antibiotic concentration is chosen to be the quality variable that

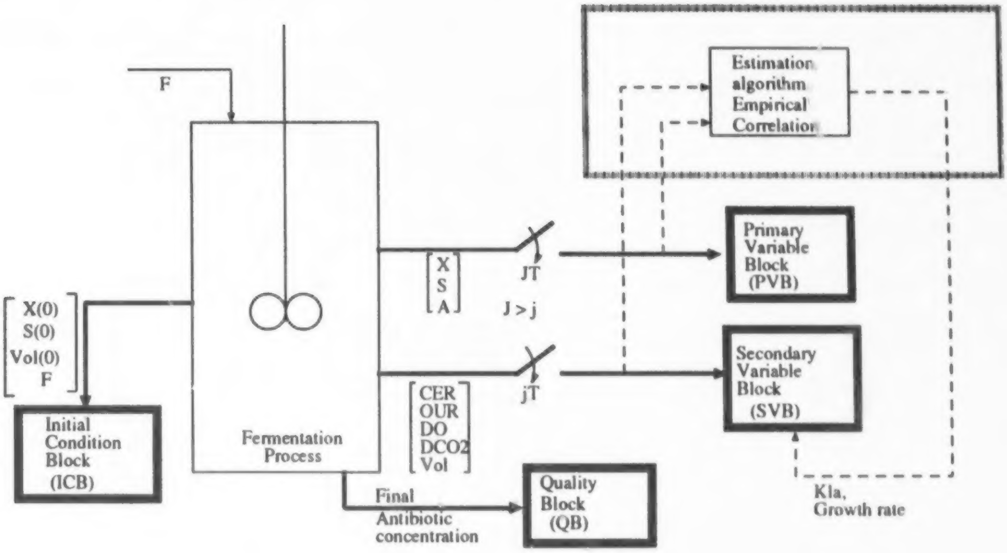

Estimation algorithm Empirical Correlation
F
Primary Variable Block (PVB)
X
S
A
JT
J > j
X(0)
S(0)
Vol(0)
F
Secondary Variable Block (SVB)
jT
CER
OUR
DO
DCO2
Vol
Initial Condition Block (ICB)
Fermentation Process
Quality Block (QB)
Final Antibiotic concentration
Kla, Growth rate

Figure 8.1 Block diagram of the measurement system for a fed-batch fermentation.

needs to be predicted on-line and forms the Quality block (QB). The primary process variables are measured infrequently and are assayed off-line. These measurements form the primary variable block (PVB). Sampling instants when the primary measurements arrive are termed as the major sampling instants. Other secondary measurements are available more rapidly from the fermentation through the use of non-invasive sensors. Typical among these are the carbon dioxide evolution rate (CER) and the oxygen uptake rate (OUR) whose values can be made available through the analysis of exit gas concentrations using an on-line mass spectrometer. The dissolved oxygen concentration is a critical process variable for an aerobic fermentation and its levels are measured using a dissolved oxygen probe. High levels of dissolved CO_2 can be inhibitory to cell growth and may reduce the production of secondary metabolites (Atkinson and Mavituna (1991)). It is not uncommon to monitor the dissolved CO_2 levels using a dissolved CO_2 probe (Puhar *et al.* (1980)). Broth volume measurements can be made available on-line on a frequent basis by the use of load cells. All these measurements constitute the secondary variable block (SVB) and the sampling instants when they arrive are termed as the minor sampling instants. The concept of analytical redundancy has been used extensively to perform fault diagnosis and isolation tasks (Frank (1990)). In the latter approach, inherent redundancies contained in the static and dynamic relationships among the system inputs and measured outputs are exploited by the use of a process model. The same concepts have been used in our work to incorporate *a priori* information about the process into the proposed monitoring scheme. For example, the production of secondary metabolites such as antibiotics is known to be significantly influenced by the initial specific growth rates (Nelligan and Calam, (1983), Calam (1987)). Regular estimates of these specific growth rates generated using an adaptive estimator (Gudi *et al.*, (1995)) can optionally be included into the SVB as an analytically redundant measurement. Likewise, estimates of the gas-liquid mass transfer coefficient K_{la}, that are normally generated on-line (Omstead *et al.* (1990)), can also be included into the SVB. The nutrient addition rate F usually follows an a priori determined/prescribed feeding policy. These rate measurements can be included into the SVB. In this work however, a constant feeding rate is assumed in the simulations and therefore the nutrient feeding rate has been included as an initial condition into the initial condition block (ICB). The ICB is formed by incorporating initial conditions on the biomass, substrate concentrations and the broth volume that are used in the fermentation. The data blocks described above can be generated for several normal batches. With the exception of the ICB and the QB, the blocks thus generated are three dimensional having the batch number as the third dimension in addition to the temporal and variable dimensions.

8.3 The M3PLS algorithm

Standard linear regression techniques (MLR, ordinary least squares) are commonly used to model the relationship between two variables. However, when several correlated variables exist for example in a fermentation, such algorithms cannot be used. The PLS algorithm is often recommended for use in such a scenario. The M3PLS algorithm proposed in this section is an extension of the standard PLS algorithm to include a third dimension viz. variation of the process variables among batches.

8.3.1 Standard PLS regression

The PLS algorithm has been proposed in several different versions (Wold(1978), Hoskuldsson,1988). The most popular version of the algorithm due to Wold (1978) is presented here. Consider the case where we have *n* measurements on *k* process variables (CER,OUR, temperatures, pressures *etc.*) and *m* quality and productivity variables (for example : antibiotic concentrations/ compositions in fermentors, molecular weight distributions and polydispersity in polymer reactors). Suppose that an inferential model that predicts the quality variables (Y space) given measurements of the process variables (X space) is desired. The process variables subspace is highly correlated because the measured variables such as the CER, OUR are all associated with the primary growth phenomenon occuring in the fermentation. Use of the ubiquitous multiple linear regression (MLR, ordinary least squares) for inferential model building can result in serious numerical problems owing to the collinear nature of the process variables (X space). In such situations, multivariate statistical techniques like PCA or PLS is often recommended to overcome problems resulting from the ill conditioned nature of the measurement space.

PLS can be viewed as a *stepwise procedure* where a pair of *maximally correlated* components (latent variables) in the X and Y spaces are selected. After the latent dimension is extracted, the X and Y spaces are deflated by removing information that has already been used up (in the case of X space) or explained (for the Y space). The extraction of latent variables is continued on the residual space as long as no significant improvement in the predictive capability of the model is achieved by including an additional PLS dimension. The deflation procedure ensures the orthogonality of the latent variables for the X and Y spaces. Also, due to the collinearity or correlation between the process variables, only a few latent variables are adequate to build a good predictive model relating X to Y. Thus, a lower dimension model building is achieved.

Remark : Simultaneous extraction of the latent variables through information exchange between the X and Y spaces and the data compression feature of PLS stand in stark contrast to ordinary least squares where neither of them occur. Consequently, it is often noticed that while MLR gives a better fit of the calibration data (because it uses all the X space information), PLS gives better predictions as it abstracts only predictive information from the dataset.

In PLS, the X and Y matrices are decomposed as :

$$X = \sum_{a=1}^{A} t_a p_a^T + E = TP^T + E \tag{8.1}$$

$$Y = \sum_{a=1}^{A} u_a q_a^T + F = \sum_{a=1}^{A} b_a t_a q_a^T + F = TBQ^T + F \tag{8.2}$$

In the above decomposition, each t_a (a=1,...A) is a score vector that indicates the relationship between the observations (samples). This means that similar observations in the X space have the same score value. p_a and q_a are the loading vectors for the X and Y spaces respectively carrying the relational information between variables of the individual matrices. The Y block scores u_a's are estimated via a linear "inner relationship", $b_a t_a$ (with b_a being the slope of the fit); this explains the relatively better predictive capabilities of the PLS model. The T, P, U and Q matrices are constructed by grouping the t, p, u and q vectors respectively. B is a diagonal matrix with the b_a's as elements. E and F denote the residuals of the X and Y spaces ; they may contain the less relevant characteristics of the data and the noise elements. The number of dimensions (A) to be retained in the final model is determined either based on the percentage sum of squares explained by the model or by the use of sophisticated and statistically sound techniques like cross-validation.

The PLS components are extracted in stages through a sequence of singular value decompositions or by an iterative technique commonly known as the nonlinear iterative partial least squares (NIPALS) algorithm. The main steps of the NIPALS technique are outlined in Table 8.1. Figure 8.2 illustrates the iterations in the NIPALS algorithm (the step numbers are included in the figure to facilitate easier understanding).

Prediction using PLS

The NIPALS algorithm can be used to generate the PLS model given by equations 8.1 and 8.2. Using this model, predictions of the Y block for new values of X can be made. The t score of the new observation X_{new} is calculated as $t_{new} = X_{new} W (P^T W^{-1})$. The value of y corresponding to X_{new} can be predicted as $\hat{y}_{new} = t_{new} B Q^T$. These predictions made using the PLS model will be good as long as the relationship between the variables at the current state does not significantly differ from that in the database of normal operations. If there are any changes made in the process (feed changes, adding a controller, *etc.*) that alter the structural relationships between the variables, it is necessary to rebuild the model (or build several models for each operating condition) for using it in a predictive mode.

For online monitoring and fault detection in process plants, a simple check on the validity

Table 8.1 : The NIPALS Algorithm

1. Start : $a=1$.
2. Set u equal to any column of Y.
3. Regress columns of X on u to get weights for X : $w^T = u^T X / u^T u$
4. Normalize w to unit length.
5. Compute the scores : $t = X w / w^T w$.
6. Regress columns of Y on t to get loadings for Y : $q^T = t^T Y / t^T t$.
7. Compute the new score vector for Y : $u = Yq / q^T q$.
8. Check convergence of u (steps 3 and 7) : if yes go to next step ; if no go to step 3.
9. Compute loadings for X by regressing columns of X on t : $p^T = t^T X / t^T t$.
10. Compute residual matrices : $R_X = X - tp^T$ and $R_Y = Y - tq^T$.
11. $a=a+1$; Append t, u, p, q and w to T, U, P, Q and W matrices respectively; Replace X by R_X and Y by R_Y and repeat steps 2 to 11 for the next PLS dimension (if needed).

In this version of the PLS algorithm, the b_a's will be unity. Further scaling of the vectors generated by the above procedure will result in b_a's that are not equal to unity. However, this does not affect any of the results.

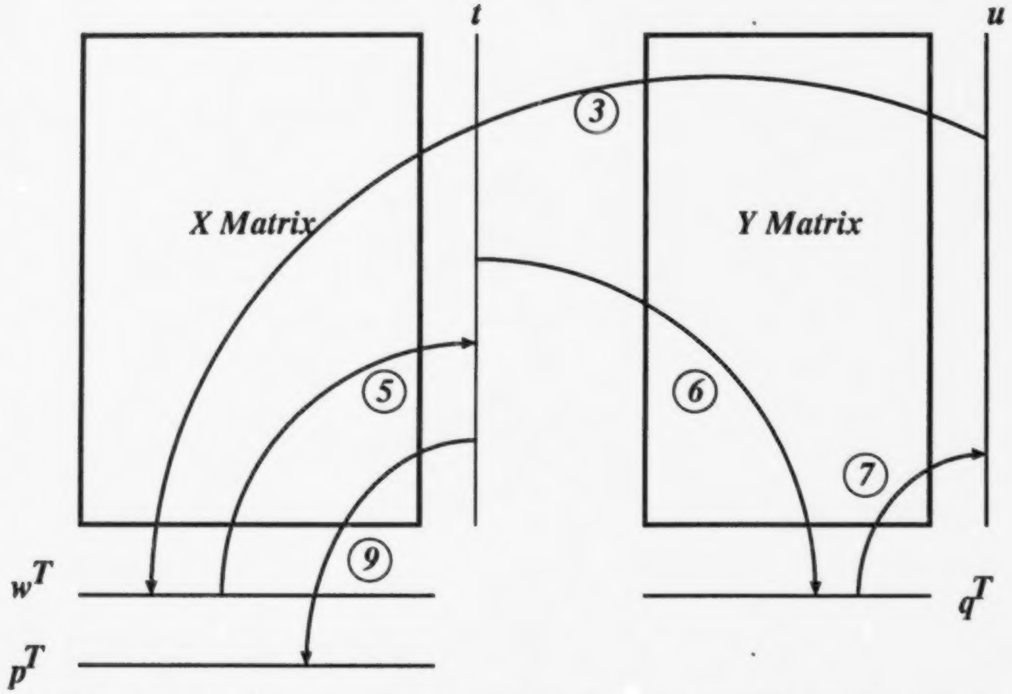

t
u
③
X Matrix
Y Matrix
⑤
⑥
⑦
⑨
w^T
p^T
q^T

Figure 8.2 : Block diagram illustration of the iterations involved in the NIPALS algorithm.

of the PLS model will show if a *statistically significant excursion* from normal plant operation has occurred. This is done (as we shall see later) using a multivariate version of the familiar univariate charting procedures like the Shewart chart. If an abnormal situation is indicated, corrective measures can be initiated or the operations halted safely.

8.3.2 Database Structure for Multirate Batch Monitoring

To build a reasonable template of normal operation for a batch process, it is necessary that a database of acceptable runs be available. Very often, the database can be built as shown in Figure 8.3. The initial conditions block is a two dimensional matrix of size NB *by* NIC where NB is the number of batch runs that make up the database and NIC is the number of variables. The quality variables block has NQ variables recorded for each batch - these variables are the quality and performance variables that are predicted online using the M3PLS model (note the arrows from all the other blocks pointing towards this block). The primary and secondary variable blocks are different in the sense that they have a higher dimension. This feature is necessary because these variables are recorded over the duration of the entire batch run for each batch. We assume that the primary block and secondary blocks carry information on NP and NS variables respectively. The key difference between these blocks is that the secondary variables are sampled more frequently compared to the primary variables. Consequently, the secondary variables block is shown as having a greater depth (NSS > NPS). For the fermentation system considered here, the variables that go into the different blocks have been detailed in Figure 8.1.

8.3.3 M3PLS Analysis

Decomposition of the 3-way matrices and the building of the PLS model to relate the quality block to the other blocks can be done in different ways. Published literature in the area of chemometrics abounds in algorithms that range from ones that are modest to those that use intricate tensor algebra (Wold *et al.* (1987), Geladi (1989) and Smilde and

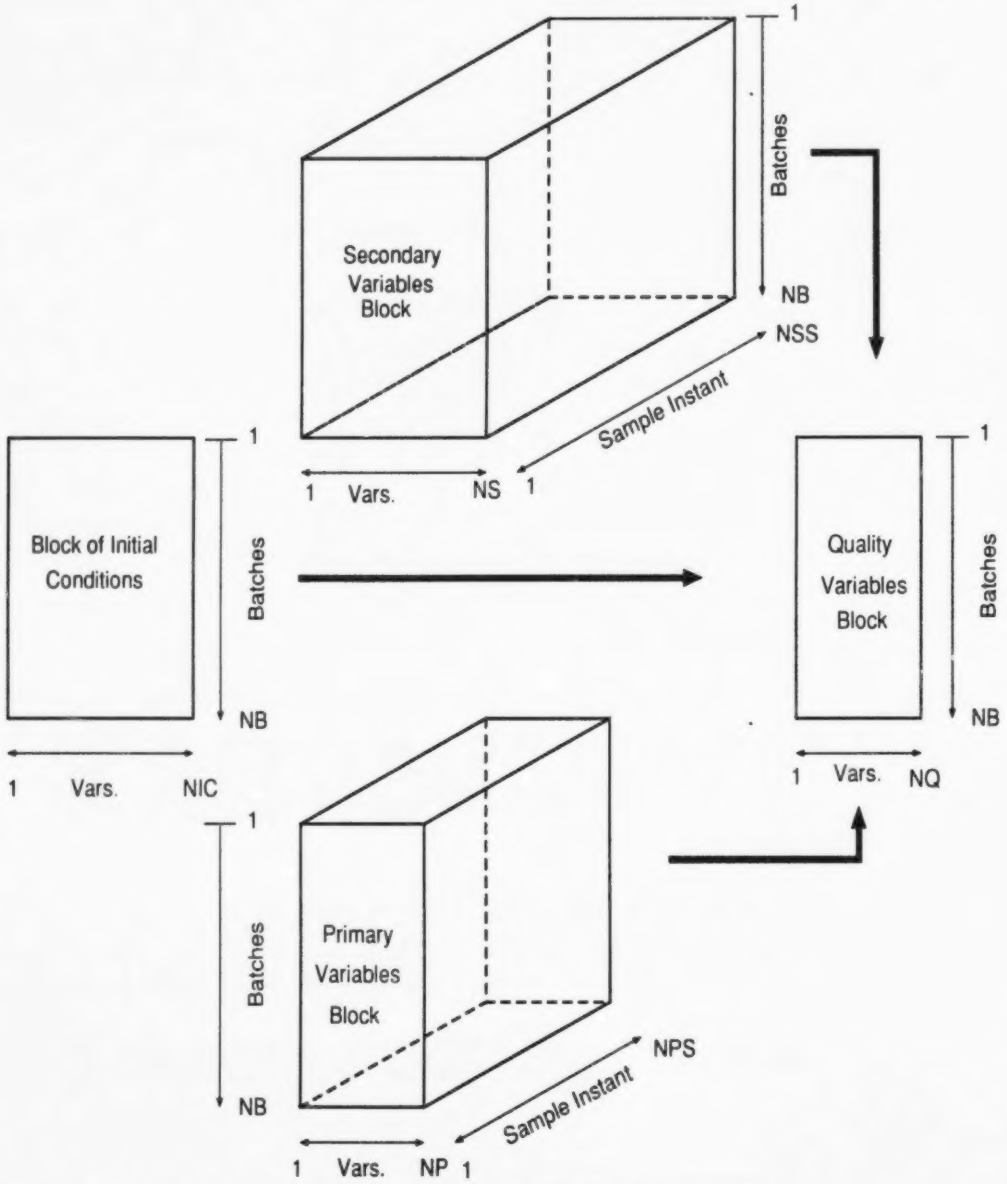

Secondary Variables Block
Batches
1
NB
NSS
Sample Instant
1
1 Vars. NS
Block of Initial Conditions
Batches
1
NB
1 Vars. NIC
Quality Variables Block
Batches
1
NB
1 Vars. NQ
Primary Variables Block
Batches
1
NB
NPS
Sample Instant
1
1 Vars. NP

Figure 8.3 : The database structure for multirate batch monitoring.

Doornbos(1991)). In this work, we will employ the multiblock PLS algorithm of Wangen and Kowalski (1988) that is simple to use and is easily interpreted as an extension of the two block PLS algorithm.

Matrix Unfolding

Using the approach of Nomikos and MacGregor (1994a), the three dimensional primary and secondary variable blocks are unfolded into two dimensional matrices by placing each of the vertical slices side by side to the right, beginning with the one corresponding to the first sampling instant for each block. The unfolding process results in two *broad* matrices of size (NB *by* (NP*NPS)) and (NB by (NS*NSS)) for the primary and secondary blocks respectively.

Data Pretreatment

Pretreatment of original data is often recommended when using PCA or PLS. These methods can be analyzed as eigenvalue-eigenvector problems and hence the magnitude of the measurements can influence the results of the PCA/PLS analysis considerably. Prior information about variables can be used to effect the scaling procedure; alternately, when little is known about the variables, autoscaling (mean centering and variance scaling) is done to give equal importance to all the variables. If certain crucial physiochemical phenomena are known to occur during a particular time interval, higher weight can be assigned to the variables in this time period by scaling them differently.

Batch processes are characterized by significant nonlinearities and the absence of a steady state. Mean centering of the initial conditions matrix, the unfolded primary and secondary matrices and the final quality block removes the nonlinearities and trends (dynamics) in process data. With this particular unfolding and autoscaling, the M3PLS model tries to characterize and relate the deviation about the mean trajectories of all the variables. In what follows, the primary and secondary variable blocks are assumed to have been unfolded and all the resulting two dimensional matrices are autoscaled. For notational simplicity, the autoscaled initial conditions matrix, primary variables matrix, secondary variables matrix and final quality variables matrix are denoted by Z_1, Z_2, Z_3 and Z_4 respectively.

M3PLS Model Development

The logical structure of the fermentation monitoring and prediction scheme alongwith its layout is shown in Figure 8.4. The M3PLS model calculation steps are outlined in Table 8.2. The iterative computational sequence to obtain the scores and loading vectors for one M3PLS dimension is depicted in Figure 8.5. Table 8.3 shows the relationship between the labels shown in Figure 8.5 and the step numbers in the M3PLS algorithm outlined in Table 8.2.

8.3.4 Monitoring and Online Predictions with the M3PLS Model

Monitoring and online predictions for a currently running batch is a nontrivial issue unlike the predictions with the standard PLS algorithm. As in the standard PLS, prediction with the M3PLS model depends on the calculation of appropriate t-scores. There is no difficulty for the initial conditions block as all the information is available in a (1 *by* NIC) vector. However, the primary and secondary variable information is not complete - at the start of the batch it is empty and gradually gets filled as the batch progresses. This means that at any time instant, *except at the end of the batch*, we need to make a pragmatic guess for the future primary and secondary variables to arrive at reasonable predictions for the final quality variables. Nomikos and Macgregor (1995) suggest three approaches to fillup the unknown data in the process variables vector. A brief summary of their guidelines is presented below :

Approach 1 : This approach assumes that the future observations are in full agreement with the mean trajectories as calculated from the reference database. This means that we fill the autoscaled values (that is used in the M3PLS algorithm) with zeros. The result is a good graphical representation of the batch run but at the cost of the t-scores being reluctant to flag an abnormal plant operation particularly at the start of a batch run.

Approach 2 : An alternate approach is to assume that the future deviations from the mean trajectories are equal to the deviations noticed at the current time. This is done at

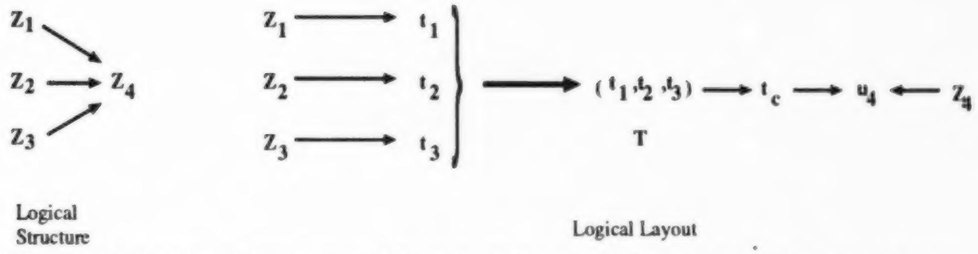

Z1
Z2
Z3
Z4
Z1
Z2
Z3
t1
t2
t3
(t1,t2,t3)
T
tc
u4
Z4
Logical Structure
Logical Layout

Figure 8.4 Logical Structure and layout of the prediction and monitoring scheme.

Table 8.2 : The M3PLS Algorithm

```
1.  Start : $a=1$.

2.  Set $u_4$ equal to any column of $Z_4$

3.  Backward Phase
    (3a) $Z_4$ predicts no blocks, so $t_4 = u_4$.
    (3b) $Z_1$, $Z_2$ and $Z_3$ predict $Z_4$.
        for $i=1$ to 3 do
        (3b1) $w_i^T = u_4Z_i$
        (3b2) Normalize $w_i$
        (3b3) $t_i = Z_iw_i$
        end

4.  Forward Phase
    (4a) $Z_1$, $Z_2$ and $Z_3$ are not predicted, so $u_i = t_i$ $(i=1,2,3)$.
    (4b) Form composite matrix : $T=[t_1\ t_2\ t_3]$.
    (4c) Compute the composite score vector $t_c$.
        (4c1) $w_c^T = u_4^TT$
        (4c2) Normalize $w_c$
        (4c3) $t_c = Tw_c$


4.  (4d) Compute updated vector $u_4$ for $Z_4$.
        (4d1) $c_4^T = t_c^TZ^4$
        (4d2) Normalize $c_4$
        (4d3) $u_4 = Z_4c_4$
```

5. Check convergence of u_4 (steps 3 and 4d3) : If yes go to next step; else go to step 3.

6. Compute predictor coefficients and loadings.
   (6a) Predictor coefficients : $b_c = t_c^T u_4 /(t_c^T t_c)$
   (6b) Loadings for Z_1, Z_2 and Z_3 : $p_i = Z_i^T t_i/(t_i^T t_i)$ (i=1,2,3)

7. Compute residuals.
   $E_i = Z_i - t_i p_i^T$ (i=1,2,3)
   $E_4 = Z_4 - b_c t_c c_4^T$. Note that u_4 is estimated as $b_c t_c$.

8. a=a+1; Append computed vectors to appropriate matrices; Repeat steps 2 to 8 and extract other M3PLS dimensions (if needed).

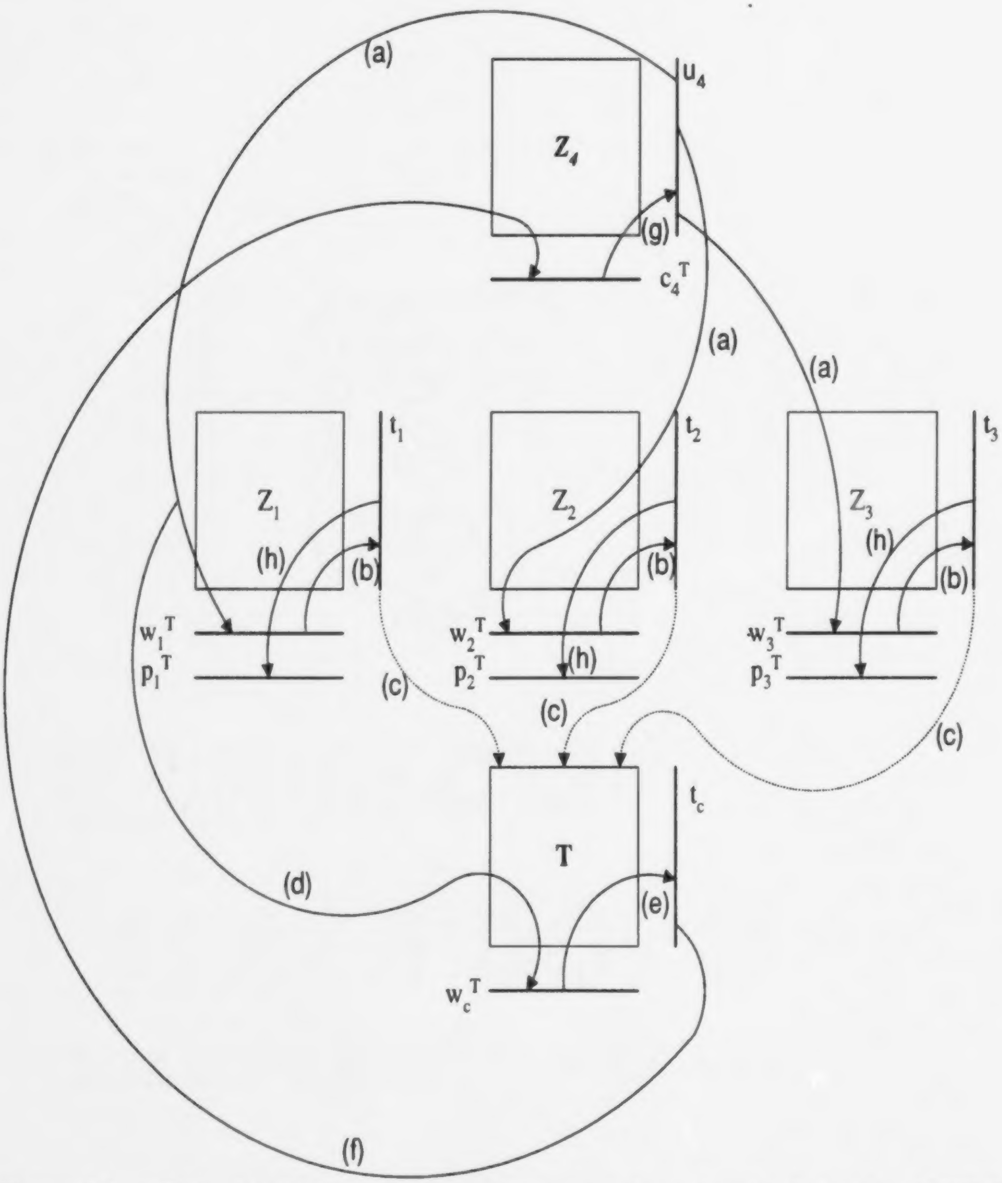

(a)
u4
Z4
(g)
c4T
(a)
(a)
Z1
t1
(h)
(b)
Z2
t2
(b)
Z3
t3
(h)
(b)
w1T
p1T
w2T
p2T
(h)
w3T
p3T
(c)
(c)
(c)
T
tc
(d)
(e)
wcT
(f)

Figure 8.5 : Data block interactions and computations involved in the M3PLS algorithm.

Table 8.3 : Relationship between the M3PLS algorithm steps and labels in Figure 8.5

Label in Figure 8.5	Step Number
a	3b1
b	3b3
c	4b
d	4c1
e	4c3
f	4d1
g	4d3
h	6b

each sampling time. With this approach, the t-scores are sensitive and pick up an abnormality more quickly.

Approach 3 : The third approach capitalizes on the ability of PCA/PLS to handle missing data. In the PCA/PLS literature, missing data can be filled up by restricting them to be consistent with the already observed values up to the current sample. This method appears superior to the other two approaches if at least 10 % of the batch history is available for it gives large and unexplainable t-scores at the beginning of the batch. Also, the control limits calculated with this approach have constant trajectories in contrast to the earlier strategies.

Nomikos and MacGregor (1995) recommend careful employment of these approaches for process monitoring depending on the nature of the process. For batch processes that : (1) do not exhibit persistent disturbances or (2) have discontinuities in the trajectories of variables, the first approach can be used. For processes that do not have frequent discontinuities in trajectories or early deviations the third approach appears to be the most suited choice. If persistent disturbances are known to occur, then the second approach is more appropriate. A combination of these methods, for example switching from the second approach to the third after the initial start-up period is also possible. Nomikos and MacGregor(1995) report that the second approach *generally* works well in most cases.

In this work, we use a slight variant of the second approach. At each time instant, the future values are set equal to the *autoscaled* values at the current sampling instant. This is easily justified because the autoscaled values (rather than the original values) are used by the M3PLS model for monitoring and predictions. With this approach the nature and contour of the monitoring charts are very much akin to those obtained from the second approach of Nomikos and MacGregor(1995).

The key steps and mathematical expressions for online monitoring and predictions proposed in this work can then be summarized as follows :

Step 1. Preparation of the data vectors

At the start of the batch, we have the complete initial conditions data which should be scaled in exactly the same way as the initial conditions in the reference database was scaled. The scaled initial conditions information is available in the 1 *by* NIC vector, Z_1. No primary and secondary measurements are available yet. The scaled primary variables vector Z_2 and secondary variables vector Z_3 of dimension 1 *by* (NP*NPS) and 1 *by* (NS*NSS) respectively are filled with zeros implying that we expect this batch run to behave just as "normally" as the runs in the database of good operations.

When each sample from the primary or secondary blocks become available, it is first scaled in the same manner as the block for the particular time instant was scaled in the reference database. This value is then inserted into the appropriate location of the vector Z_2 or Z_3 as is the case. The values for the *remaining* entries in Z_2 or Z_3 are also set equal to the scaled values computed at the present sampling instant. In any case, we therefore have *complete* Z_1, Z_2 and Z_3 vectors at each major and minor sampling instant.

Step 2. Calculate scores for blocks Z_1, Z_2 and Z_3

$\hat{t}_1 = Z_1W_1$

$\hat{t}_2 = Z_2W_2$

$\hat{t}_3 = Z_3W_3$

Step 3. Get squared prediction error (SPE) for the initial condition matrix, primary and secondary variable block. These are useful for online monitoring as we shall see later.

Reconstruct Z_1, Z_2, Z_3 using the computed loadings $\hat{Z}_i = \hat{t}_i\, p_i^T$ (i=1,2,3)

Compute prediction errors $e_i = Z_i - \hat{Z}_i$ (i=1,2,3)

Extract the deviations for this time instant and store it in e_i (i=1,2,3)

Compute $SPE_i = e_ie_i^T$ (i=1,2,3)

Step 4. Compute combined block score

$\hat{t}_c = (\hat{t}_1 * \mathrm{diag}(W_c(1,:))) + (\hat{t}_2 * \mathrm{diag}(W_c(2,:))) + (\hat{t}_3 * \mathrm{diag}(W_c(3,:)))$

Step 5. Predict scores for quality block, z_4

$\hat{u}_4 = \hat{t}_c * \mathrm{diag}(b_c)$

Step 6. Predict quality block

$$\hat{z}_4 = \hat{u}_4 C_4^T$$

Step 7. As a final step, rescale z_4 to get *raw* predictions for the quality block.

Control Charts and Confidence Intervals

With the score vectors computed at each sampling instant (major as well as minor) and the predicted values for the final quality variables, it is possible to construct control charts for the score vectors of each block and the final quality variables. The SPE values computed for the blocks at each time instant can be used in a similar manner to perform online monitoring and fault detection.

Choice of the method used to generate missing data for new runs must be made before the control limits for the control charts are derived. This is because the same strategy should be used for handling missing data as well as generating control limits. Any change in the policy of treatment of missing observations will render the computed control limits obsolete. To derive the control limits, every batch in the database of normal runs is sent through the monitoring scheme described above as if it were a current operation. The initial condition scores and its corresponding SPE are obtained initially. At each major and minor sampling instant, the t-scores and the SPE for the secondary and composite blocks as well as the predictions of the final quality variables are collected. Primary block t-scores and the SPE are evaluated at the major sampling instants when the primary measurements arrive. Such an approach provides the external reference distribution (for each block and at each time interval) and facilitates calculation of the control limits. In doing so, it is assumed that the external distribution sufficiently captures the inherent variations observed in the database of acceptable process operations and will be applicable to assess new batch runs.

Different practical problems require the use of various types of intervals. Monitoring of future batch runs is an analytical study (Deming, 1975) as it involves the use of data from an existing process to predict the future evolution of a similar process. The use of *prediction intervals* is thus most appropriate. Prediction intervals to contain one or more future observations, or some function of the future observations, can be constructed from a previously sampled process. In our case, the external reference distribution serves this purpose. Since the reference data was collected during the normal operation of the plant (when variability in plant data can be attributed only to "common causes" rather than "assignable causes"), the prediction intervals computed often provide reasonable insights on the *health* of a future run.

A prediction interval for a single future observation is an interval that will, with a specified degree of confidence, contain the next sample from the process. Assuming that the reference as well as the future data are random samples from the same parent population (having identical production procedures and similar process conditions), the prediction intervals may be computed (Hahn and Meeker, 1991). A two sided 100(1-α)% prediction interval to contain the mean of a future, independently and randomly selected observation, using the reference data containing an independent random sample of size n from the same process described by a normal distribution, is (*cf.* equation (4.2) of Hahn and Meeker, 1991)

$$[UCL,LCL] = \bar{x} \pm t_{(1-\frac{\alpha}{2},n-1)} \sqrt{(1+\frac{1}{n})}\ S \tag{8.3}$$

where UCL and LCL refer to the upper and lower control limits respectively, $\bar{x}$ denotes the estimated mean and *S* is the standard deviation computed from the reference distribution. The factor $t_{(1-\alpha/2,n-1)}$ represents the critical values of the Student's t-distribution for a specified degree of freedom and confidence. Charting quadratic forms such as the squared prediction errors (SPE's) require the computation of one-sided confidence limits. Equation (8.3) is suitably modified and used for this purpose.

Intervals for the predicted quality variables are obtained from a regression analysis viewpoint. Computation of these involve a clear understanding of the theory of statistical linear models and concepts like estimable functions and generalized inverses. A clearer discussion of the issues involved is presented in Searle(1982). Nomikos and MacGregor(1994b) and Phatak *et al.*(1993) provide confidence intervals on final quality variables; the former is less accurate but the limits are easily computed and therefore used here. The confidence limits for the predicted *univariate* y at the significance level α with *dof* degrees of freedom at each sampling instant are given by :

$$[UCL,LCL] = \hat{y} \pm t_{(\frac{\alpha}{2},dof)} \sqrt{(MSE)} \sqrt{(1+\hat{t}_c^T(T_c^T T_c)^{-1}\hat{t}_c)} \tag{8.4}$$

In the above equation ŷ refers to any one of the predicted quality variables in z_4; $\hat{t}_c$ is the composite t scores computed for the current batch, $\hat{T}_c$ is the composite T matrix formed using the database of normal batches. MSE is the mean sum of errors computed from the database of normal operations and is given by

$$MSE = \frac{(y-\hat{y})^T(y-\hat{y})}{dof} \tag{8.5}$$

As stated earlier, the values of the above variables are to be computed or retrieved at every sampling instant to generate the confidence intervals at each instant.

8.4 Results and discussion

The M3PLS algorithm was verified using simulations involving the fed-batch fermentation described earlier using the model presented in Appendix A. The model equations adequately describe the substrate inhibition effects on the biomass growth and antibiotic production through the use of different empirical models. They however do not model the dependence of the antibiotic production rate on the specific growth rates for which no models are proposed in the literature. Due to the presence of significantly different time scales (the gas phase dynamics are significantly faster than the broth phase dynamics), a stiff differential equation solver LSODES (Hindmarsh (1983)) availabe at the Lawrence Livermore Laboratories was used to solve the model equations. The profiles of various primary and secondary process variables were generated over a time period of 120 hours of fermentation. Regular estimates of the specific growth rate μ and the mass transfer coefficient K_{la}, generated using an online estimator (Gudi *et al.*(1995)) and an empirical correlation (Omstead *et al.*, (1990)) respectively, were included into the secondary variable block. The profiles of the secondary process variables that constitute the SVB were sampled every 30 minutes while the primary process variables in the PVB were sampled every 150 minutes. White gaussian noise with zero mean was added to the measurements to simulate noisy measurements with a relatively smaller signal to noise ratio. A total of 47 simulations incorporating the common batch to batch variations were performed by using different initial conditions to yield a database of normal batch runs. The datasets in this database were used to build a template of normal process operation. The monitoring algorithm was first evaluated by presenting online data from an average normal fermentation run. The following faults or deviations that are commonly encountered in fermentations were implemented in the simulations and the resulting datasets were presented to the algorithm to evaluate its capability to perform on-line fault detection and quality prediction.

1. **Initial conditions** : Lower biomass concentrations and antibiotic yields resulting from smaller initial concentrations of actively growing biomass were simulated and used to analyze the predictive capability of the algorithm.

2. **Contamination by foreign microorganisms** : An additional contaminant state, to characterize the biomass evolved due to the growth of the foreign microorganism, was introduced into the fermentor equations. The contaminant microorganism was assumed to grow at a constant specific growth rate that was higher than the maximum specific growth rate that could be attained in the normal fermentation (Chattaway and Stephanopoulos, 1988). The foreign microorganism affected the normal fermentation profiles by taking up nutrients, oxygen and by evolving carbon dioxide. It also affected the final antibiotic titre through its effect on the environmental variables(see equation (A-10) in Appendix A). The concentration of the contaminant microorganism also showed up in the offline measurements of the biomass.

3. **Sparger disturbances** Changes in the environmental variables, such as the CER, OUR

and dissolved oxygen, resulting from sparger disturbances were simulated by manipulating the gassed power P_g (*cf.* equation A-11 in Appendix). A disturbance was introduced at 20 hours of fermentation time and removed after 23 hours of fermentation. The fermentation operation returned gradually to the normal operating regime at about 50 hours of fermentation.

8.4.1 Model Building

Table 8.4 shows the batch variations modelled by each addition principal component for each of the blocks. It can be seen that three latent dimensions are needed to model almost all of the variation in the quality block. The model building steps that result in the data shown in Table 8.4 can be interpreted according to each latent dimension as follows. The first dimension models 22.3 % , 33.3 % and 42.5 % of the variations in the initial conditions, primary variable and secondary variable blocks respectively. The first latent dimension effectively explains 47.3 % variation in the quality block. The data for the second and third latent dimensions can also be interpreted in the same way. The inner relationship plots shown in Figures 8.6 and 8.7 explain the relationship between the quality and composite blocks as modelled by the first and third latent dimensions. Almost all of the batches used in building the normal operation database fall in the linear region indicating that the inner relationship is linear. It also indicates that, for a good and normally operating batch, good predictions of the final quality variable(s) can be obtained. The inner relationship plot for the first dimension in Figure 8.6 shows a small scatter along the diagonal straight line indicating that this dimension is only partly useful in predictions of the quality block. For the third dimension relationship shown in Figure 8.7, almost all of the batches fall along a linear region indicating that this dimension is very useful in the quality predictions. The correlation coefficient, which is a linear measure of dependence between the quality and the composite blocks, is also shown in the plots in Figures 8.6 and 8.7. These plots thus emphasize that the inner relationship between the quality block and the composite block can be extracted using a linear model. If the value of the correlation coefficient was small or if the inner relationship plots had shown a

Table 8.4

Model Building Step for the M3PLS algorithm

Dimension number	% explained in IC block	% explained in PVB	% explained in SVB	% explained in the QB
1	22.31	33.33	42.50	47.30
2	50.10	60.92	47.70	84.13
3	76.36	63.21	50.25	97.52

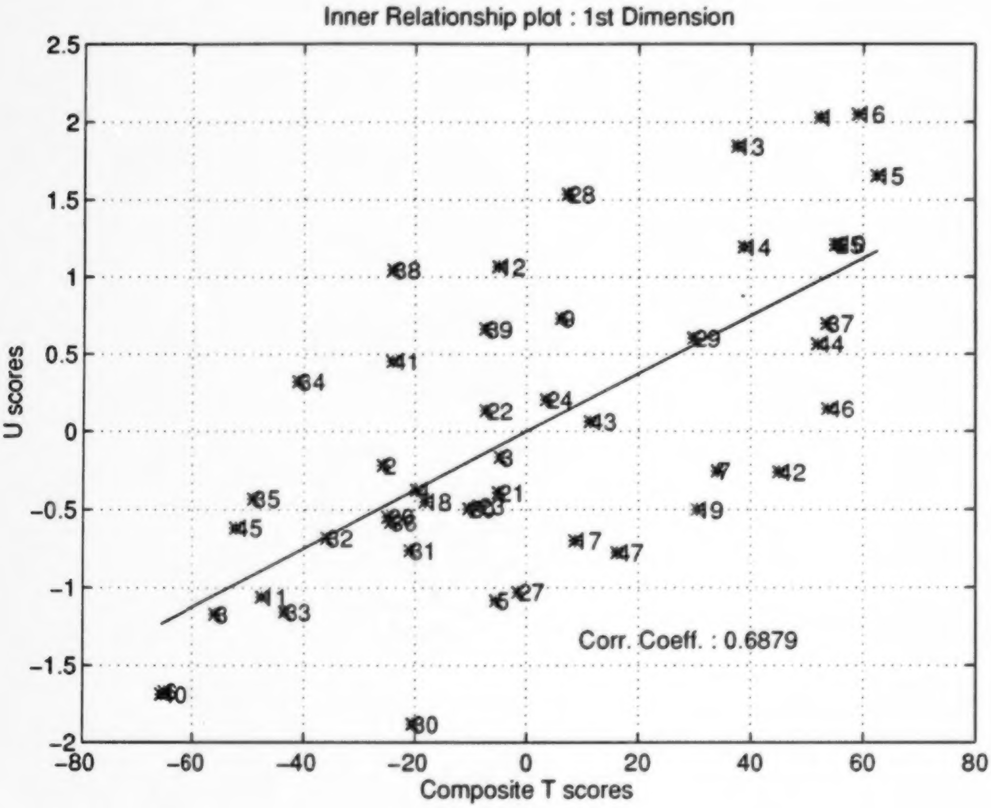


Figure 8.6 : Model building : Inner relationship plot for the first dimension. A scatter is seen along the linear region indicating that this dimension is only partly useful in quality prediction.

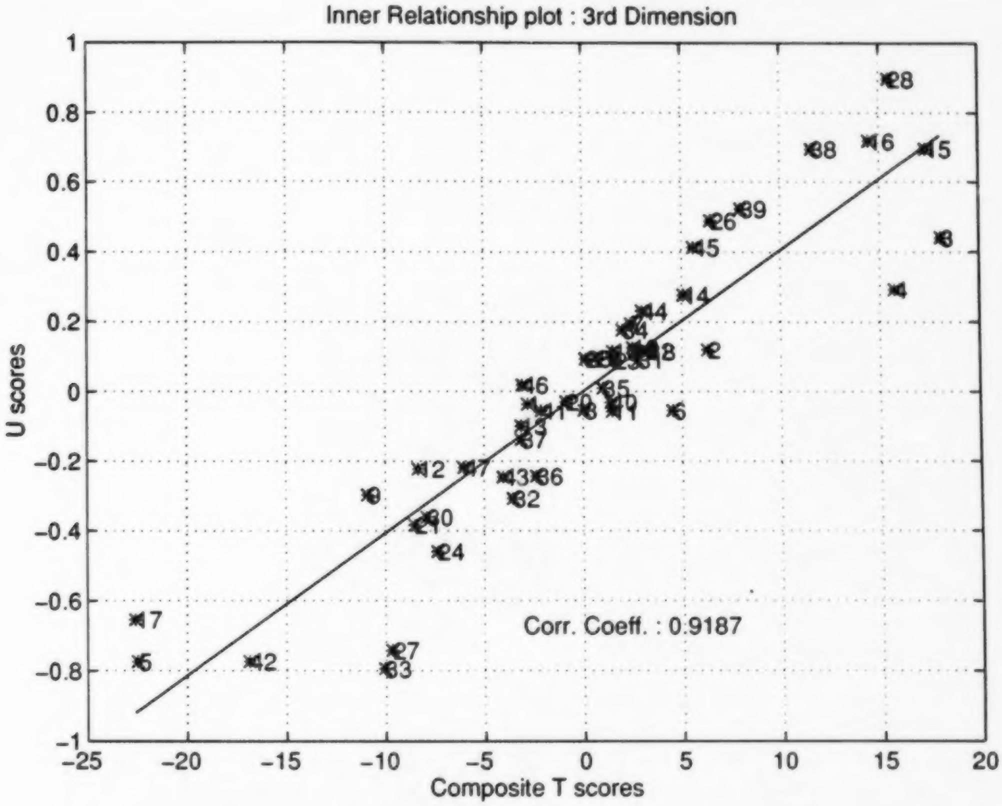


Figure 8.7 : Model building : Inner relationship plot for the third dimension. Most of the batches fall along the linear region indicating that this dimension is very useful in final quality prediction.

nonlinear trend, it would be necessary to use a nonlinear model to explain the relationship between the quality and composite blocks. From Table 8.4 it is seen that the first threedimensions explain the quality block almost completely. For the primary and secondary blocks, Figures 8.8 and 8.9 show the cumulative variation explained by each of the latent dimensions at each sampling instant. It is seen that three latent dimensions adequately explain the variation in the primary and secondary variable blocks and addition of another latent dimension is not likely to improve the modelling of the blocks significantly.

8.4.2 Monitoring a normal fermentation run

A new fermentation run, to depict normal process operation, was simulated by using the mean value of the initial conditions specified for the 47 template runs. Figure 8.10 shows the scores and the squared prediction error (SPE) plots in the initial condition block for such a normal run. It is seen that the initial condition, when projected onto the scores plots, falls in the normal region of the space spanned by the 1st and 3rd dimension scores. Also the SPE for this initial condition falls within the 99 % and 95 % control limits constructed from the database of normal batches. This indicates that one could expect the initial condition to propagate and evolve a normal, acceptable batch run. The spaces spanned by the 2nd and 3rd latent dimensions also needs to checked to ensure that the projected initial condition falls in the normal acceptable region. However, the SPE plots generally show up any abnormalities and it is therefore adequate to look at the scores plots for fewer latent dimensions with the SPE plots.

Figures 8.11 and 8.12 show the evolution of the batch as monitored by the first dimension scores and the SPE for the primary and secondary variable blocks respectively. The 99 % and 95 % upper and lower control limits generated from the database of normal plant operations is also shown and it is seen that the scores and the SPE plots of the batch that is being monitored fall within the respective control limits indicating that the fermentation run is indeed normal. Figure 8.13 shows the online final quality prediction at each sampling instant. It can be seen that the predictions made by the algorithm are quite close

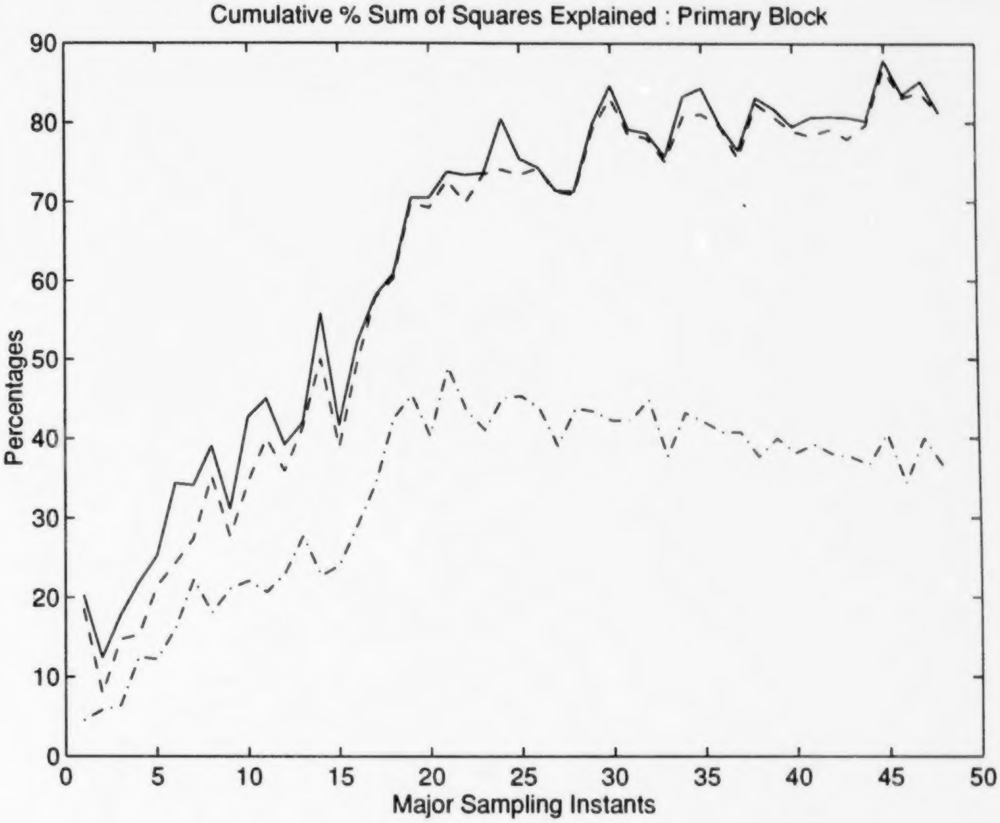


Figure 8.8 : Model building step for the primary block. The cumulative sum of squares explained for the primary block by the first ('-.'), second ('--') and third (solid line) is seen. An additional dimension is not necessary because the third dimension does not increase the % sum of squares explained.

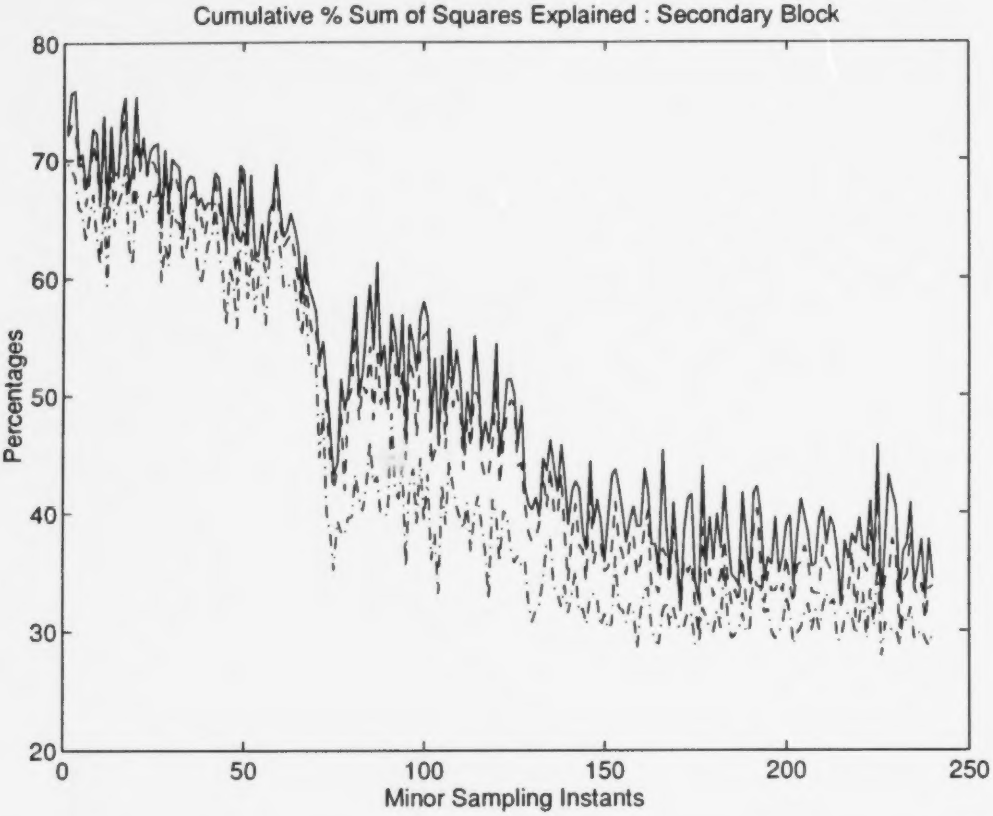


Figure 8.9 : Model building step for the secondary block. The cumulative sum of squares explained for the secondary block by the first ('-.'), second ('--') and third (solid line) is seen. An additional dimension is not necessary because the third dimension does not increase the % sum of squares explained.

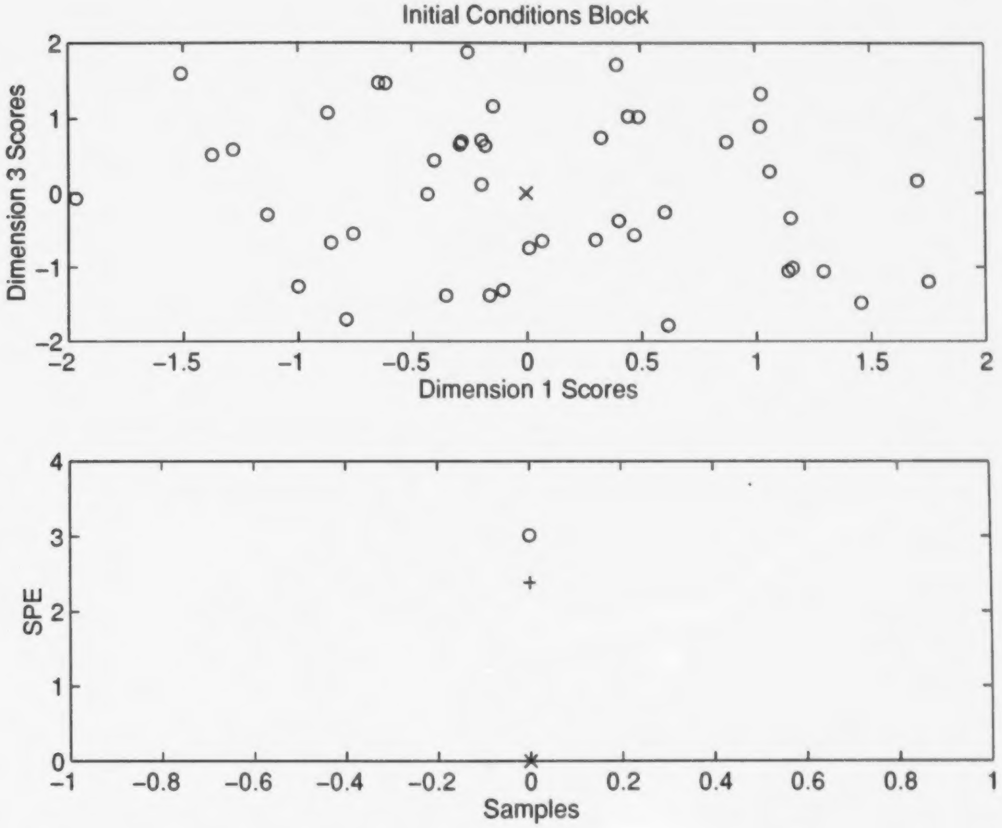


Figure 8.10 : Monitoring of a normal fermentation run via the IC block scores: The initial condition for the batch is characterized as normal in the third dimension scores. The current batch is shown by the 'x'. In the SPE plots, the 99 % limit ('o') and the 95 % limit ('+') are shown and the SPE for the current batch (shown by 'x') lies within these limits.

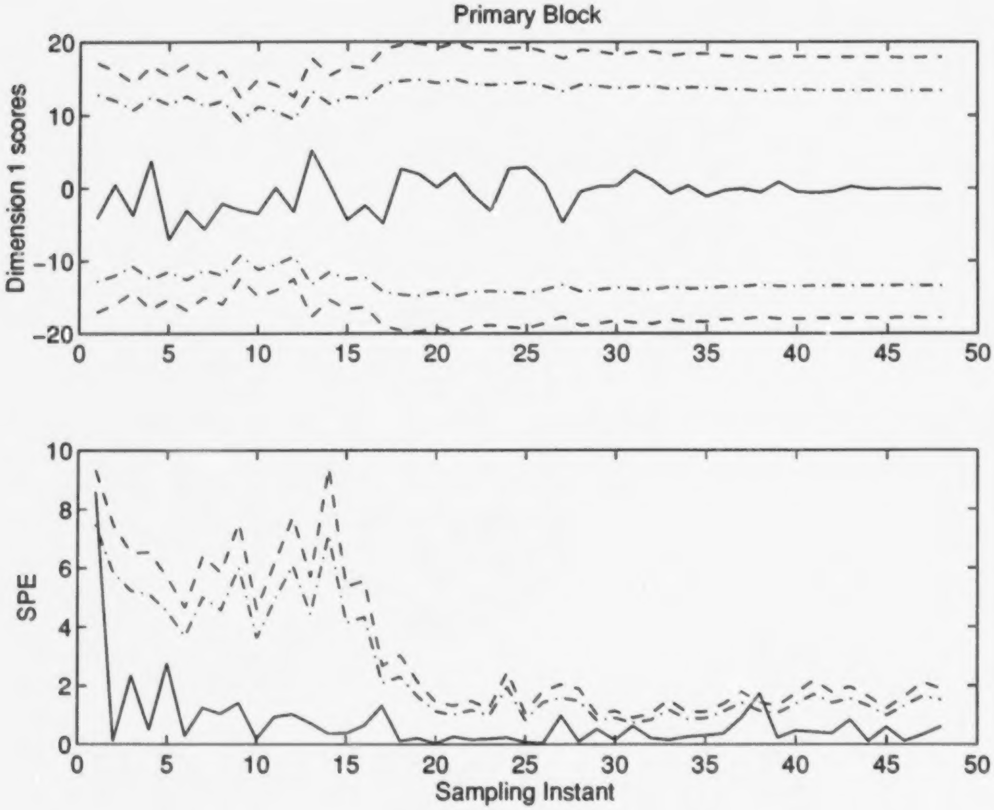


Figure 8.11 : Monitoring of a normal fermentation run via the primary block scores : The first dimension scores plot for the primary block (solid line) is seen to lie between the 99 % ('--') and 95 % ('-.') limits. In the SPE plots also, the 99 % limit ('--') and the 95 % limit ('-.') are shown and the SPE for the current batch (solid line) lies within these limits.

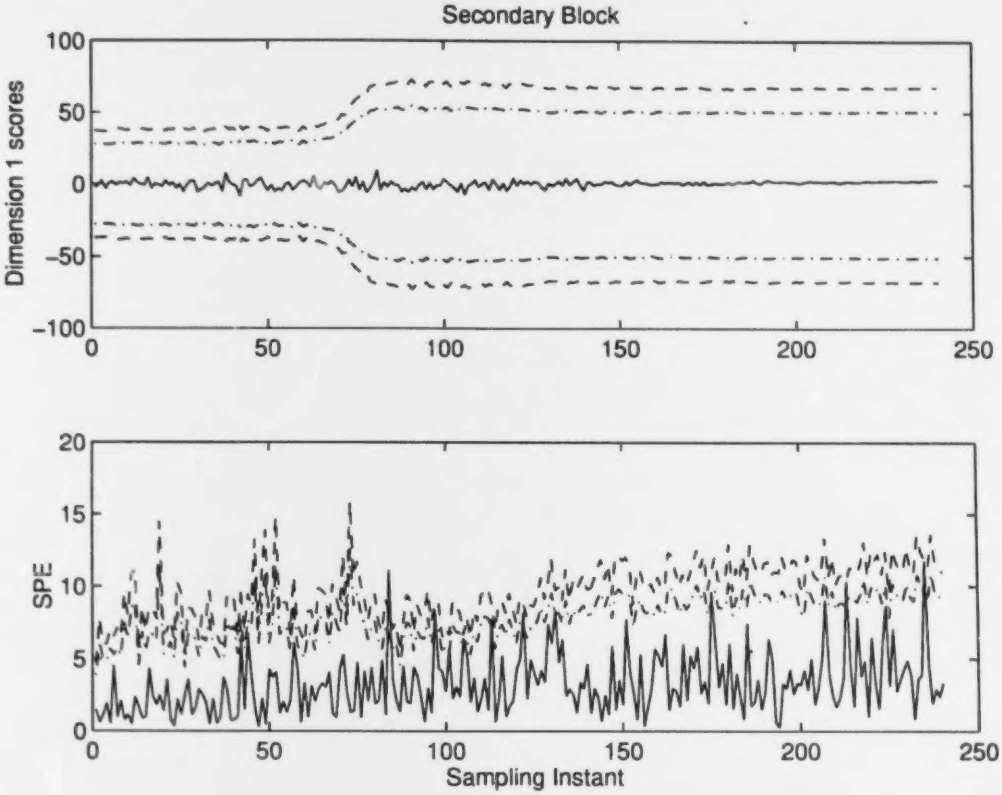


Figure 8.12 : Monitoring of a normal fermentation run via the secondary block scores : The first dimension scores plot for the secondary block (solid line) is seen to lie between the 99 % ('--') and 95 % ('-.') limits. In the SPE plots also, the 99 % limit ('--') and the 95 % limit ('-.') are shown and the SPE for the current batch (solid line) lies within these limits.

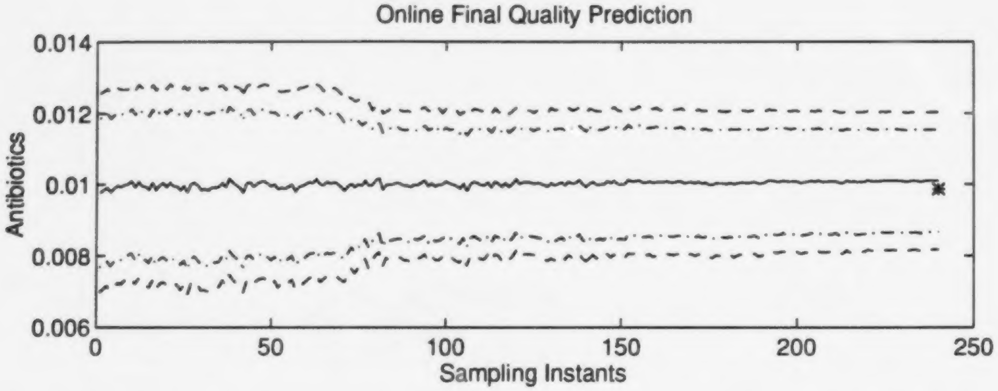


Figure 8.13 : Quality prediction of a normal fermentation run : The 99 % ('--') and 95 % ('-.') prediction limits are shown and the prediction of the final quality variable at each sampling instant is shown. The predictions agree well with the actual final value ('*').

to the actual final quality for this batch (shown by asterisk).

8.4.3 Monitoring of abnormalities in the fermentation

(i) **Abnormalities in Initial conditions** A simulation run starting with a low concentration of actively growing biomass was generated and used to evaluate the monitoring algorithm. The algorithm detects the initial condition abnormality almost immediately as seen in the scores and the SPE plots of Figure 8.14. The abnormality shows up by characterizing the batch as a distinctly different batch as seen in the third dimension scores. It also clearly shows up in the SPE plot as it falls significantly beyond the 95% and 99% control limits. Often times, the inoculum quality may be poor i.e. it may contain the right amount of biomass but a smaller proportion of actively growing biomass(Webb and Kamat, (1992)). In such a case, the abnormality may not necessarily show up in the initial conditions block. It would however manifest in the primary and secondary measurements and show up in the scores plots for the latter. Figures 8.15 and 8.16 show the first dimension scores and SPE plots in the primary and secondary blocks for the abnormal batch run. Significant deviations from the template of normal operation, as defined by the control limits, is seen. Figure 8.17 shows the profile of the online final quality predictions. It is seen that qualitative trends leading to lower antibiotic concentration are predicted online from about 30 hours of fermentation (around the 60th sampling instant in Figure 8.15). This is because the antibiotic expression gets triggerred on around this time and the measurements play a role in the final quality prediction. However, these predictions are not anywhere close to the actual value of the final quality variable. The reason for this was discussed earlier in section 8.3.1. The predictions made by the PLS model will be good only as far as the relationship between the variables does not differ significantly from that in the database of normal runs. More specifically, the model gives reasonably accurate predictions only for runs that are in control as defined by the database of normal operations. When statistically significant deviations occur, the underlying statistical model is no longer representative of the process and therefore the final quality predictions will not be accurate.

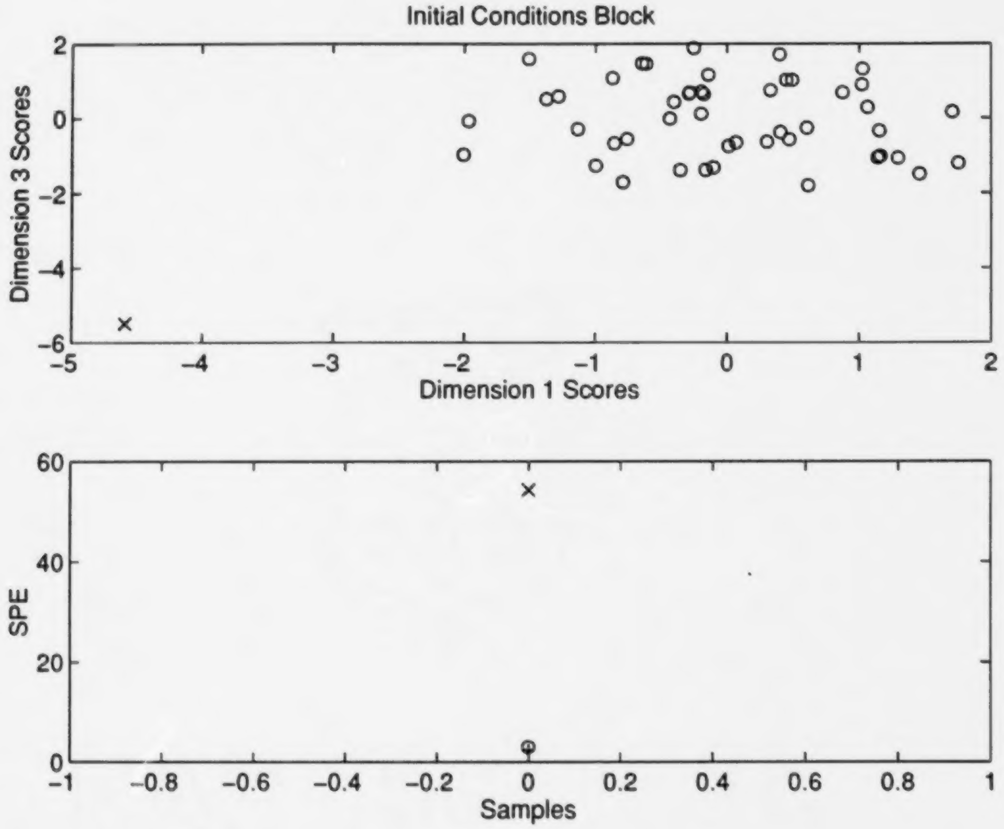


Figure 8.14 : Monitoring of abnormality in an initial condition via the IC block scores: The third dimension scores plots clearly shows the current batch('x') to be different than the normal batches('o'). The SPE plots also flag the initial condition abnormality. The SPE for the current batch ('x') lies significantly above the 99 % ('o') and 95 % ('+') limits.

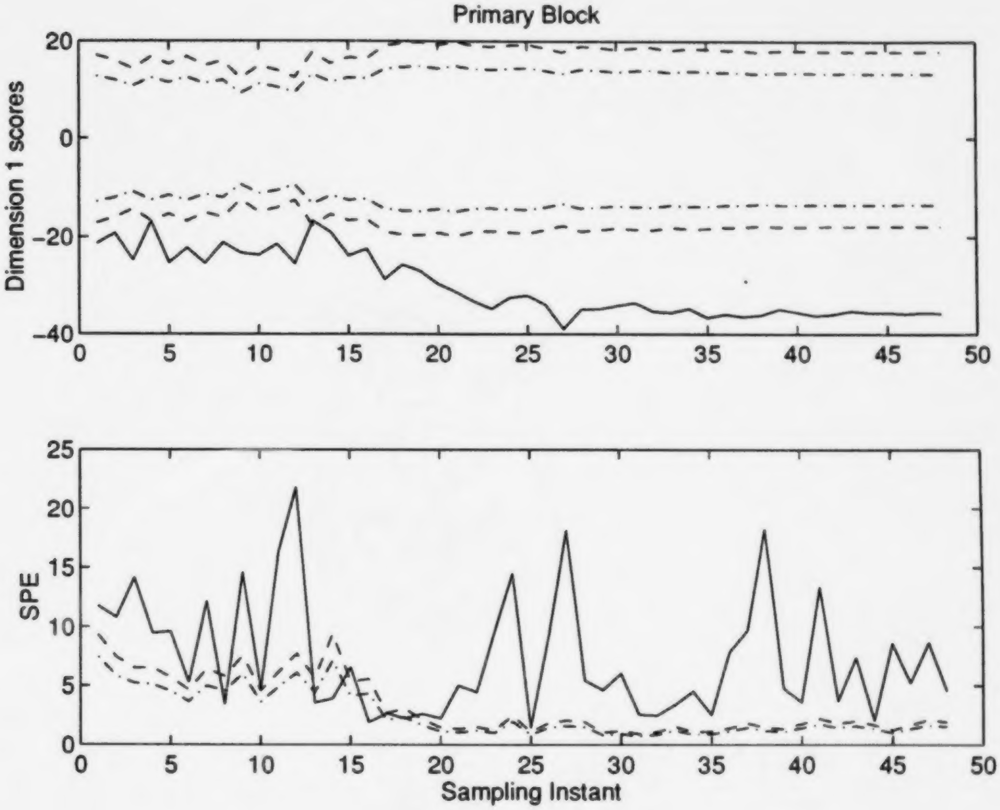


Figure 8.15 : Monitoring of abnormality in an initial condition via the primary block scores : The first dimension scores plots for the primary block clearly shows the current batch(solid line) to be abnormal as it lies outside the 99 % ('--') and 95 % ('-.') limits. The SPE plots also flag the abnormality. The SPE for the current batch (solid line) lies significantly above the 99 % ('--') and 95 % ('-.') limits.

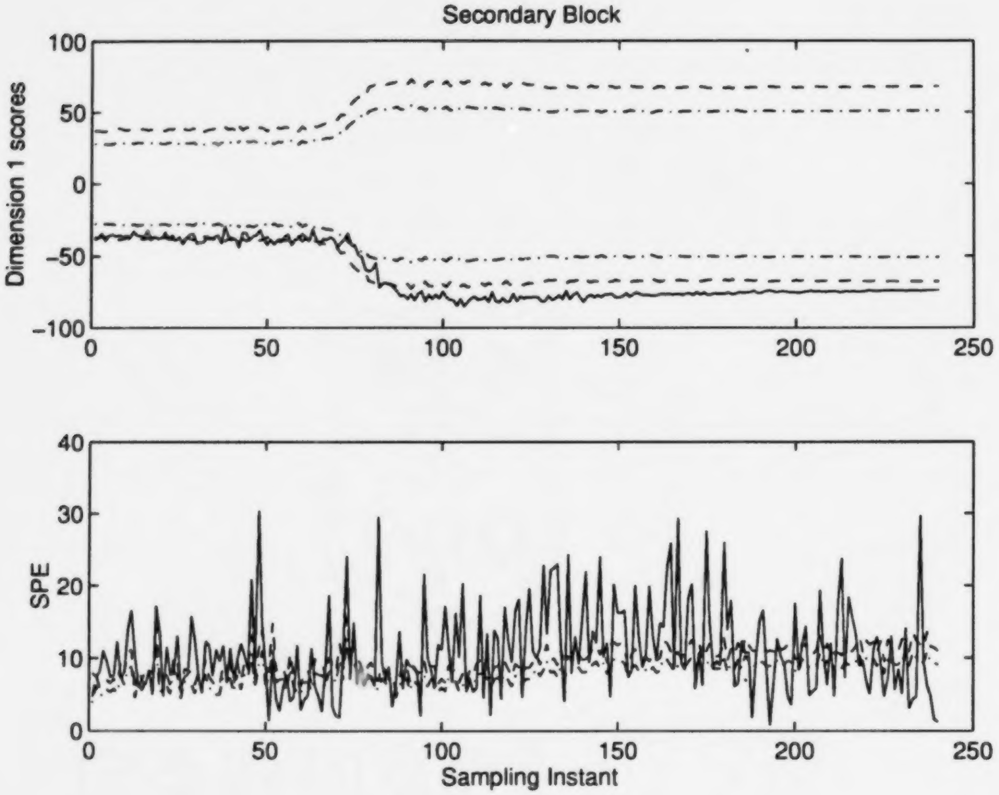


Figure 8.16 : Monitoring of an abnormality in an initial condition via the secondary block scores : The first dimension scores plots for the secondary block clearly shows the current batch(solid line) to be abnormal as it lies outside the 99 % ('--') and 95 % ('-.') limits. The SPE plots also flag the abnormality. The SPE for the current batch (solid line) lies significantly above the 99 % ('--') and 95 % ('-.') limits.

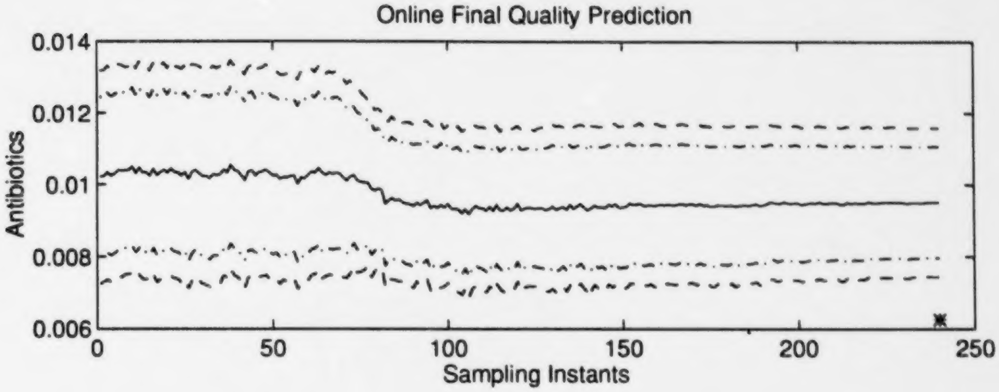


Figure 8.17 : Quality prediction for a batch having an abnormality in an initial condition: The online final quality prediction is seen along with the 99% ('--') and 95 % ('-.') prediction limits. The decreasing trend in the final quality predictions is seen around the 70th sampling instant due to the arrival of the actual measurements of the quality variable. The predictions (solid line) do not agree with the actual value of the final quality ('*').

(ii) **Abnormalities in the sparging system** Starting from a normal initial condition, disturbances were introduced in the sparging system at 20 hours of fermentation. This corresponded to the 40th sampling instant for the secondary block (secondary measurements arrive every half hour). Figures 8.18 and 8.19 show the first dimension scores and the SPE for the secondary and the combined composite blocks respectively. It is seen that the abnormalities show up quickly in the scores plots. The scores trajectory for the abnormal run is seen to deviate from the template of normal operations till the disturbance is withdrawn at 23 hours of fermentation. The trajectory gradually returns into the normal region after the disturbance is withdrawn. Since the deviations from the template of acceptable plant operation is not significant, the final quality prediction in Figure 8.20 is close to the actual value in the simulations. It must be emphasized here that this example of abnormality is chosen only to illustrate the monitoring capability of the algorithm. In an actual fermentation, the occurrence of a disturbance and its subsequent compensation will not necessarily bring the fermentation into the normal region of operation. The switching to a different metabolism of glucose and its effect on product yields in a recombinant fermentation, under dissolved oxygen fluctuations, considered by Namdev *et al.* (1993) is an excellent illustrative example of this phenomenon.

(iii) Abnormality due to foreign microorganism contamination

Starting from a normal initial condition, a contaminant microorganism was introduced at 40 hours of fermentation and assumed to grow at a constant specific growth rate of 0.8 hr^{-1}. This corresponds to the 80th sampling instant for the secondary variable block. Figure 8.21 shows the first dimension scores plot and the SPE for the secondary block. It is seen that deviations in the process operation is detected almost immediately. This is also seen in the composite scores plot of Figure 8.22. The monitoring of this batch was terminated at 50 hours of fermentation due to the runaway nature of the monitored profiles.

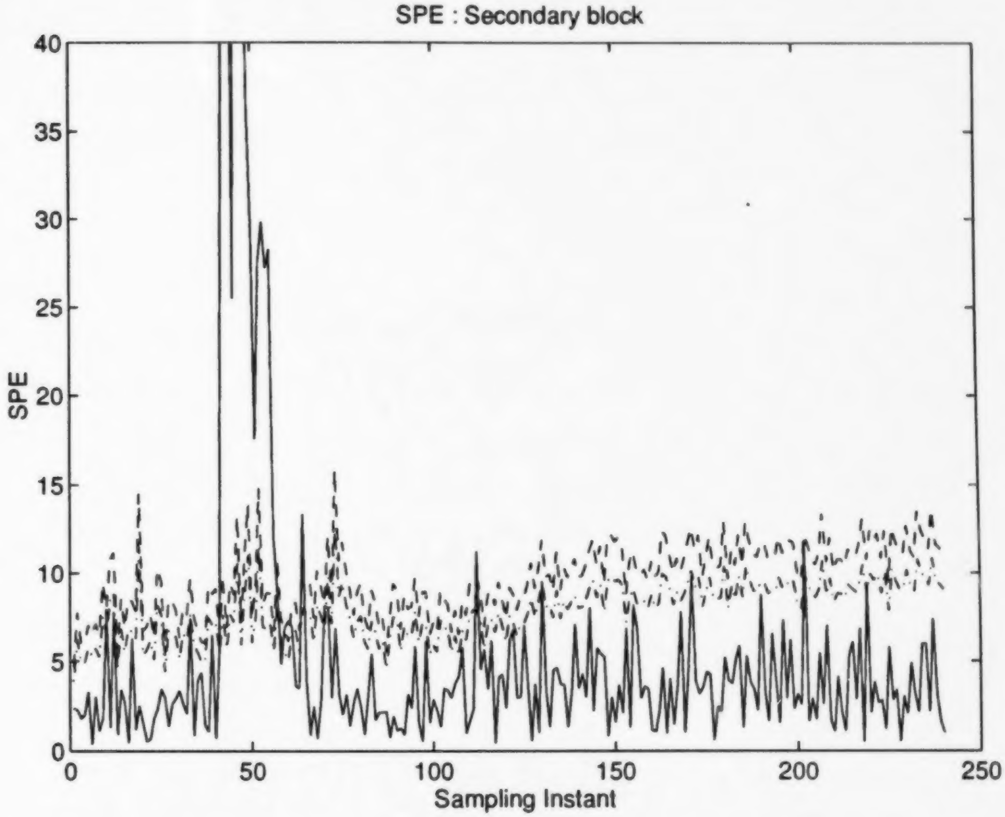


Figure 8.18 : Monitoring of an abnormality in the Sparging system via the SPE plots: The SPE plots for the current batch deviate from the 99 % ('--') and 95 % ('-.') limits when the disturbance is applied but return to the normal region when the disturbance is removed.

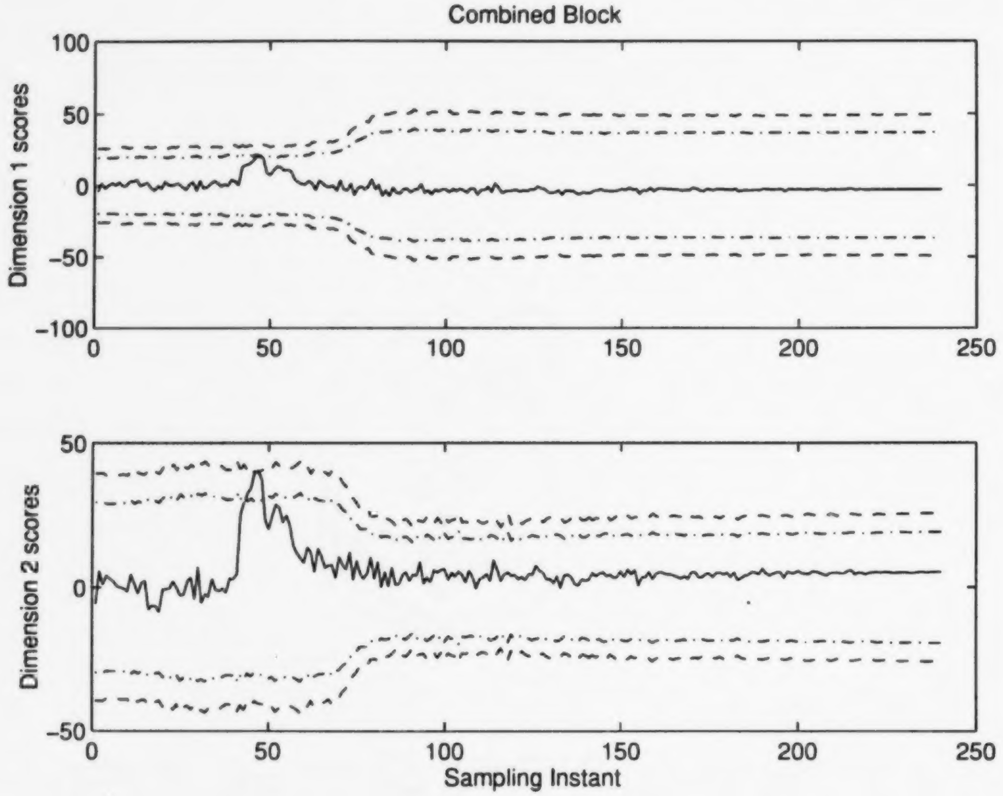


Figure 8.19 : Monitoring of an abnormality in the Sparging system via the secondary block scores: The scores plots for the current batch as monitored by the first and second latent dimensions deviate and approach the 99 % ('--') and 95 % ('-.') limits when the disturbance is applied but return to the normal region when the disturbance is removed.

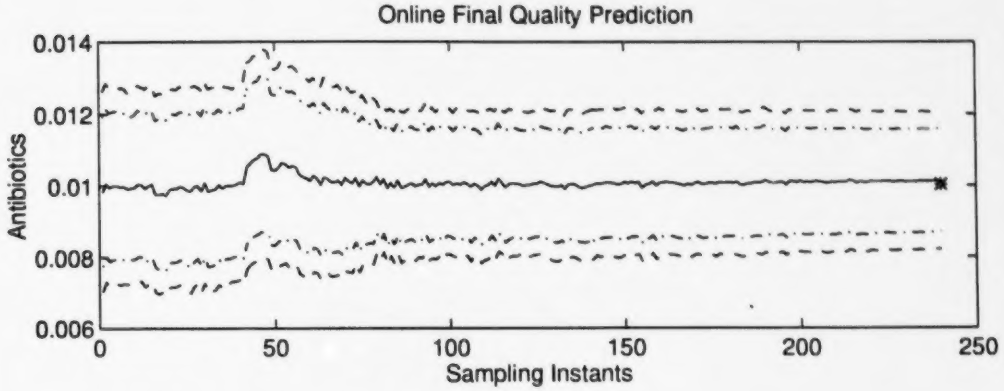


Figure 8.20 : Quality prediction of a batch with abnormality in the Sparging system : The final quality prediction does deviate slightly when the disturbance is applied. However, since the disturbance is not sustained for a long time, the final quality predictions are good.

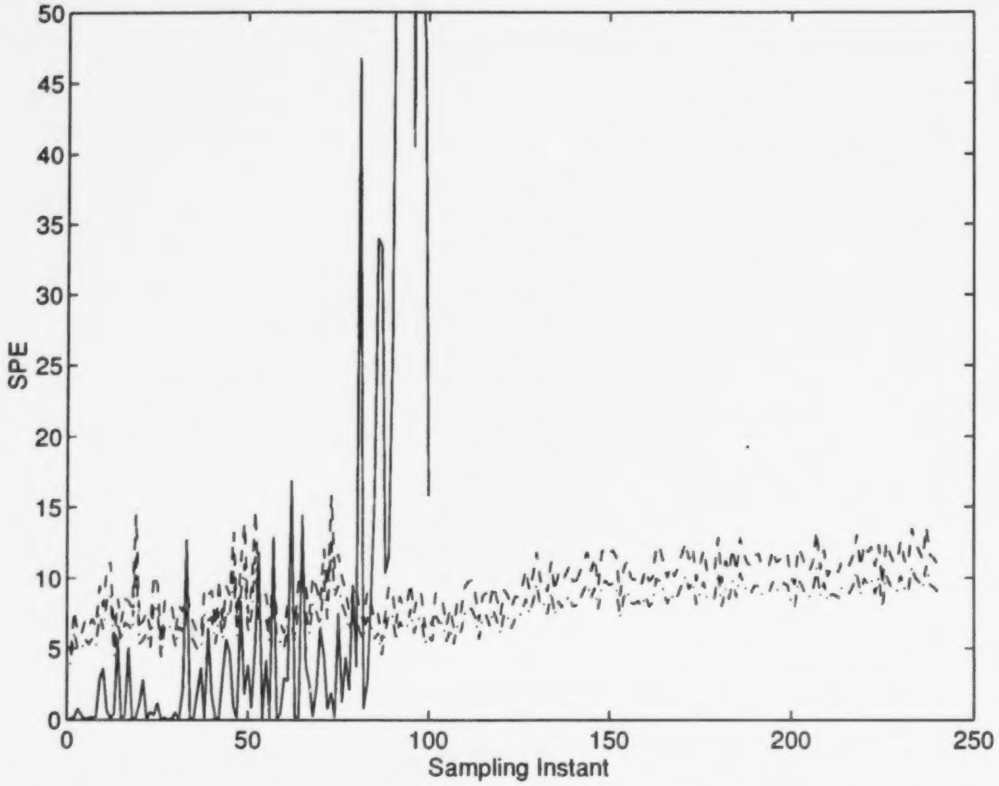


Figure 8.21 : Detection of contamination of a batch by foreign microorganisms via the SPE plots : The SPE plots (solid line) deviate from the 99 % ('--') and 95 % ('-.') limits. The monitoring was stopped at the 100th sampling instant.

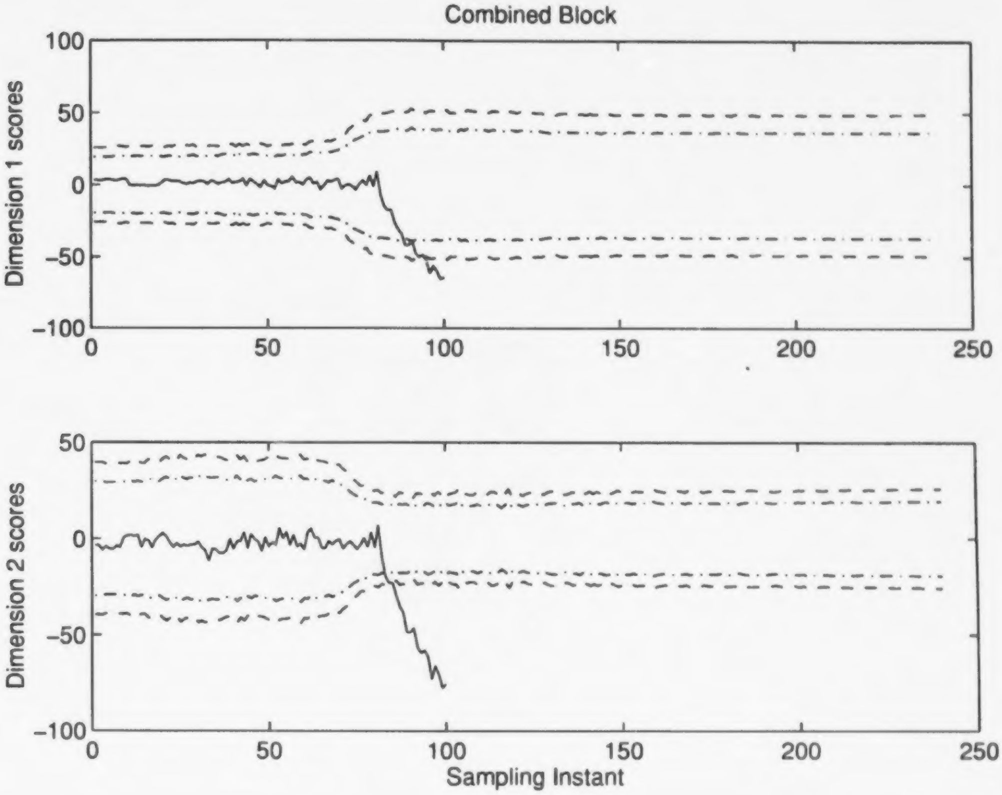


Figure 8.22 : Detection of contamination of a batch by foreign microorganisms via composite block scores: The combined/composite block scores plots (solid line) deviate from the 99 % ('--') and 95 % ('-.') limits. The monitoring was stopped at the 100th sampling instant.

8.5 Conclusions

The multirate, multiway, multiblock PLS (M3PLS) algorithm proposed in this work has been evaluated for the monitoring of fed batch fermentation processes. The algorithm is quite simple in its implementation. It only requires a database of normal plant operating data. The computationally intensive part of the algorithm is performed only once during the model building step. The monitoring of a new batch run using the statistical model is relatively easy to implement online. The algorithm achieves data reduction and makes the monitoring task easy as fewer variables in the transformed space need to be monitored. The monitoring algorithm gives quick online flagging of abnormal batch runs. It also gives good online predictions of the final quality variable for new batches as long as the batch has no abnormalities or deviations from the normal behaviour.

Although the PLS based strategies proposed in this work and elsewhere in literature offer excellent potential as monitoring algorithms, they are perhaps not appropriate for the task of final quality prediction. For a good normal run as characterized by the SPE and scores plots, the range in which final quality value would lie is usually known. For an abnormal run, the underlying statistical model is obsolete and does not give good final quality predictions. Although the effects of an abnormal run and subsequent trends in the final quality variable can be qualitatively seen, the predictions are far from accurate due to the invalidity of the statistical model in the presence of significant deviations from normal plant behaviour. In this sense, the utility of the algorithm for final quality prediction is rather limited and not of much practical value. An alternative, useful approach could be to implement such algorithms in a multiple model framework to characterize online highly productive, intermediate and least productive batches based on the scores plots. This has been done preliminarily using a principal component analysis (PCA) based algorithm (Saner and Stephanopoulos, (1992)) and can be implemented elegantly in a M3PLS framework. This would enable quick and online characterization of the batches so as to implement remedial operations when possible and necessary.

8.6 References

Atkinson, B. and Mavituna, F., Biochemical Engineering and Biotechnology Handbook, Stockton Press, New York, USA (1991).

Calam, C.T., "Process development in Antibiotic Fermentations", Cambridge Studies in Biotechnology 4, (1987).

Deming, W.E, " On Probability as a basis for action", The American Statistician, **29**, p146-152, (1975).

Frank, P.M., "Fault diagnosis in Dynamic Systems using Analytical and Knowledge based redundancy. A survey and some new results", Automatica, **26**, p459-474, (1990).

Geladi, P., "Analysis of Multi-Way (Multi-Mode) Data", Chemometrics and Intelligent Laboratory Systems, **7**, p 11-30, (1989).

Gudi, R.D., Shah, S.L., and Gray M.R., " Adaptive Multirate Estimation strategies with application to a bioreactor", Accepted for publication in the AIChE Journal, (1995).

Hoskuldsson, A., " PLS Regression methods", J. Chemometrics, 2, p211-228, (1988).

King,R., "Early detection of Hazardous states in Chemical Reactors", Dynamics and Control of Chemical Reactors and Distillation Columns", IFAC symposium, Bournemouth UK, (1986).

King,R., "Plant and Sensor Failure detection by multiple nonlinear filters", In: Proceedings of Modelling and Control of Biotechnical Processes 1992 and 5th International Conference on Computer Applications in Fermentation Technology,Keystone, Colorado,(Eds) Karim M.N and Stephanopoulos G , Pergamon Press, New York (1992).

Kresta,J., MacGregor, J.F., and Marlin, T.E., "Multivariate Statistical monitoring of Process operating performance", Canadian Journal of Chemical Engineering, **69**, p35-47, (1991).

MacGregor, J.F., Jaeckle,C., Kiparissides, C. and Koutoudi, M.," State Estimation for Polymerization ", IFAC Symposium on Dynamics and Control of Chemical

Reactors and Distillation Columns, Bournemouth UK, Pergammon Press, New York, USA(1986).

Namdev, P.K., Irwin, N.C., Thompson, B.G., and Gray, M.R., " Effects of dissolved oxygen fluctuations on recombinant E-coli fermentation", Biotechnology and Bioengineering, **41**, p666-670, (1993).

Nelligan,I. and Calam, C.T., " Optimal control of Penicillin Production using a minicomputer", Biotechnology Letters, **5**, p 561-566, (1983).

Nomikos,P., and MacGregor, J.F., "Monitoring of Batch Processes using Multi-way Principal Component Analysis", AIChE Journal, **10**(8), p1361-1425, (1994a).

Nomikos,P., and MacGregor, J.F., "Multi-Way Partial Least Squares in Monitoring Batch Processes", Internet Conference on Chemometrics,(1994b).

Nomikos,P. and MacGregor, J.F.," Multivariate SPC Charts for monitoring Batch Processes", Technometrics, **37**(1), p 41-59, (1995).

Omstead,D.R., Phillips J.A., and Humphrey, A.E., "Indirect Parameter Estimation", In : "Computer Control of Fermentation Processes", Omstead D.R. (Ed), CRC Press, Inc., Boca Raton, Florida, USA (1990).

Phatak,A., Reilly, M., Penlidis, A., "An Approach to Interval estimation in PLS regression", Analytica Chimica Acta, **277**, p495-501, (1993).

Puhar E., Einsele A., Buhler H. and Ingold W., "Steam sterilizable pCO_2 electrode" Biotechnology and Bioengineering, **22**, p2411-2416, (1980).

Saner, U. and Stephanopoulos, G., "Application of pattern recognition techniques to fermentation data analysis", In: Proceedings of Modelling and Control of Biotechnical Processes 1992 and 5th International Conference on Computer Applications in Fermentation Technology,Keystone, Colorado,(Eds) Karim M.N and Stephanopoulos G , Pergamon Press, New York, USA (1992).

Searle, S.R., "Matrix Algebra Useful for Statistics", Wiley Publishing Co., New York, USA (1982).

Smilde, A.K, and Doornbos, D.A., "Three-Way methods for the calibration of chromatographic systems : comparing PARAFAC and Three-way PLS", Journal of Chemometrics, **5**, p345-360 (1991).

Venkatsubramamanian, V. and Chan, K.," A Neural network methodology for Process Fault Diagnosis", AIChE Journal, **35**, p1993-2002, (1989).

Wangen, L.E., and Kowalski, B.R., "A Multiblock PLS Algorithm for investigating complex chemical systems", Journal of Chemometrics,**3**, p3-20,(1988).

Webb, C. and Kamat, S.P., "Improving fermentation consistency through better inoculum preparation", Biopractice (1992).

Wold, S., Geladi, P., Esbensen, K. and Öhman, J., "Multi-Way Principal Components and PLS-Analysis", Journal of Chemometrics, **1**, p41-56, (1987).

Wold, S., "Cross-Validatory estimation of the number of components in factor and principal component models", Technometrics, **20**, p397-405, (1978).

8.7 Nomenclature

p	loading vector for the process variables space
q	loading vector for the process variables space
t	scores vector for the process variables space
u	scores vector for the quality variables space
E	residual matrix in the process variables space
F	residual matrix in the quality variables space
NB	number of batches in the database
NIC	number of initial condition variables
NQ	number of quality variables
NP	number of primary variables
NS	number of secondary variables
P	loading matrix constructed by appending loading vectors (X block)
Q	loading matrix constructed by appending loading vectors (Y block)
T	scores matrix constructed by appending scores vectors (X block)

U	scores matrix constructed by appending scores vectors (Y block)
W	matrix of weights (see Table 8.1)
X	matrix of process variables
Y	matrix of quality variables
Z_1	unfolded, autoscaled initial condition block
Z_2	unfolded, autoscaled primary variable block
Z_3	unfolded, autoscaled secondary variable block
Z_4	unfolded, autoscaled quality variable block

Superscripts

^	estimates
-	mean
c	composite block

Acronyms

CER	carbon dioxide evolution rate
OUR	oxygen uptake rate
PCA	principal component analysis
PLS	projection to latent structures
M3PLS	multiway, multiblock, multirate PLS
QB	quality block
PVB	primary variable block
SVB	secondary variable block
ICB	initial condition block
MLR	maximum likelihood regression
NIPALS	nonlinear iterative PLS
UCL	upper control limit
LCL	lower control limit

SPE	squared prediction error.

Chapter 9

Conclusions and Recommendations

9.1 Conclusions

The direct and most important result of the research carried out in this thesis is a formal way of incorporating delayed, off-line primary variable measurements into online adaptive estimation, regulation and statistical monitoring schemes. The contributions developed in the chapters of this thesis are outlined in the conclusions at the end of each individual chapter. However, the following conclusions are presented in a logical order to link and consolidate the various contributions of this research.

The multirate formulation of the classical Kalman filter, that was proposed by Glasson (1983) for use in aerospace applications, was found to be useful in formally accommodating the multiple rates of measurement availability commonly found in chemical and biochemical processes.

Structural observability issues were found to be very important for estimator design.

Measurement delays were found to reduce system observability and adversely affect estimator performance. The structural observability analysis performed symbolically helped to modify the measurement system so as to enhance system observability in the presence of measurement delays in the primary process outputs.

The adaptive estimation task for the case of multiple rates of output sampling was performed using the multirate extension of the classical Kalman filter. In the first approach, the multirate Kalman filter is used with a least squares parameter estimator to perform the task of state and parameter estimation in a sequential framework. The second approach used the extended Kalman filter in a multirate framework to simultaneously estimate the states and parameters from the measurements. It was found that issues related to measurement delays in the primary process variables were addressed more elegantly in the multirate EKF framework. This approach also required relatively smaller tuning effort when compared with the sequential approach.

The adaptive state estimator as implemented in the multirate EKF framework, was used to successfully generate estimates of critical states and parameters in an experimental fed-batch antibiotic fermentation. Effects of endogenous metabolism and changing maintenance activity on the system observability and estimator performance was systematically analyzed. The system equations were approriately modified to enhance system observability.

System observability problems were also encountered while performing the task of nutrient concentration estimation. A reduced order estimator proposed in the literature (Stephanopoulos and San,(1984)), was used in a multirate framework and was found to yield accurate estimates of the nutrient concentrations and the substrate to biomass yield coefficient. The resulting adaptive estimator was extensively validated using simulations and industrial data.

A new formulation of the projection to latent structures (PLS) algorithm was used to incorporate off-line quality measurements into online statistical batch process monitoring schemes. The resulting strategy was validated extensively using simulations.

9.2 Recommendations for future work

1. This thesis proposes to alleviate reduced system observability due to measurement delays by appending past inferential measurements. The estimates generated in this fashion can be refined further by using a fixed or variable lag smoother. Mutha *et al.* (1994) have used a fixed lag smoother for estimating states from delayed measurements in a polymerization reactor. The smoothing algorithm can be implemented in a multirate Kalman filter framework.

2. Observability problems in substrate estimation have been encountered because models proposed in the literature, to describe the influence of substrate concentrations on the specific growth rate, are not adequate and hence are not used. An alternative approach, especially in an adaptive context, could be to model the influence of substrate and biomass concentrations on the specific growth rate through the relationship :

$$\mu = k_1 X - k_2 S \tag{9.1}$$

and then estimate k_1 and k_2 online. This approach has been preliminarily evaluated and found to give promising results. However, this approach needs to be evaluated further in more depth.

3. Product concentrations can also be estimated online using an empirical model that relates the product expression rates to growth and non-growth associated components (Roels, 1983). An *ad-hoc* assumption for the time delay in the appearance of the product can be made based on observed, past fermentation runs. This approach has been preliminarily evaluated and found to be promising. Secondary product expression is triggered on when substrate concentrations falls below inhibitory values. So, an alternative approach to model the time delay in the product expression can be based on substrate concentration estimates.

4. Product expression rates are known to be influenced by initial growth rates (Calam, (1987)). If a high initial growth rate profiles is followed, the precursors necessary for the product expression reactions are not synthesized. If a lower growth rate profile is followed, lower biomass concentrations are seen. Thus, there appears to be an optimal value of the initial growth rate that gives optimal rates of secondary product expression. Since the multirate state estimator generates accurate estimates of the specific growth rate, it could be used at a process development stage to arrive at optimal values of the specific growth rate.

5. There has been considerable recent interest in integrating statistical monitoring schemes with process control schemes (Mertens *et al.*(1994)). These ideas need to be investigated and developed in the context of bioprocess engineering.

9.3 References

Calam C.T., "Process development in Antibiotic fermentations", Cambridge Studies in Biotechnology, (1987).

Mertens B., Thomson M. and Fearn T., " Principal Component Outlier detection and SIMCA: A synthesis", The Analyst, **119** (12), 1994.

Mutha R.K., Cluett W.R. and Penlidis A., " Efficient State Estimation for a Polymerization reactor with multirate measurements", Canadian Society for Chemical Engineering Conference, Calgary, October (1994).

Roels, J.A., " Energetics and Kinetics in Biotechnology", Elsevier Biomedical Press, Amsterdam, The Netherlands (1983).

Stephanopoulos G. and San K.Y., " Studies on on-line bioreactor identification", Biotechnology and Bioengineering, **26** , p 1176-1188, (1984).

APPENDIX-A

Dynamic balances to simulate a fed-batch fermentation

Biomass

$$\frac{dX}{dt} = r_X - \frac{X}{V_L} F \quad ; X(0)=1.05 \ (\frac{g}{l}) \tag{A.1}$$

Substrate

$$\frac{dS}{dt} = -\frac{r_X}{Y_{X/S}} - \frac{r_P}{Y_{P/S}} + \frac{S_F}{V_L}F - m_S\frac{S}{K_m+S}X - \frac{S}{V_L}F \ ; S(0) = 0 \ (\frac{g}{l}) \tag{A.2}$$

Secondary product

$$\frac{dP}{dt} = r_P - k_d P - \frac{P}{V_L}F \ ; P(0) = 0 \ (\frac{g}{l}) \tag{A.3}$$

Dissolved Oxygen

$$\frac{dO_L}{dt} = k_L a(O_L - O_L^{*}) - m_o X - \frac{r_X}{Y_{X/O}} - \frac{r_P}{Y_{P/O}} - \frac{O_L}{V_L}F \ ; O_L(0) = 1d\text{-}03 \ (\frac{mmol}{l}) \tag{A.4}$$

Dissolved carbon dioxide

$$\frac{dC_L}{dt} = k_L a(C_L^{*} - C_L) + m_C X + \frac{r_X}{Y_{X/C}} + \frac{r_P}{Y_{P/C}} - \frac{C_L}{V_L}F \ ; C_L(0) = 33 \ x \ 10^{-5}(\frac{mmol}{l}) \tag{A.5}$$

Oxygen in gas phase(volume fraction)

$$\frac{dO_g}{dt} = \frac{RT}{\varepsilon p}\left[-K_{la}(O_l^{*} - O_l) + \frac{pq_{in}[O_{in} - O_g]}{RTV}\right]; \ O_L^{*} = p\frac{O_G}{H_o} \ ; O_g(0) = 0.21; \tag{A.6}$$

Carbon dioxide in gas phase(volume fraction)

$$\frac{dC_g}{dt} = \frac{RT}{\varepsilon p}\left[-K_{la}(C_l^{*} - C_l) + \frac{pq_{in}[C_{in} - C_g]}{RTV}\right]; \ C_L^{*} = p\frac{C_g}{H_c}; \ C_g(0) = 0 \ ; \tag{A.7}$$

Fermentor volume

$$\frac{dV_L}{dt} = F \ ; V_L(0) = 3 \ (l) \tag{A.8}$$

Growth Model

$$r_X = \mu_X \frac{S}{K_X X + S} \frac{O_L}{K_{OX} O_L^{*} X + O_L} X - kd_1 \ \mu_X \ X \tag{A.9}$$

Product Formation Model

$$r_P = \mu_P \frac{S}{K_P + S(1 + \frac{S}{K_I})} \frac{O_L^p}{K_{OP} X + O_L^p} \tag{A.10}$$

Rheological properties

$$k_L a = (1.0+2.8N_i)\left(\frac{1000P_g}{V_L^3}\right)^{a'}(v_s)^{b'}(v_{app})^{c'} \tag{A.11}$$

$$v_{app} = v_0 + \alpha X^{2.5} \tag{A.12}$$

Nominal values of the parameters in the simulation model

a'	$k_L a$ correlation exponent (0.9)
b'	$k_L a$ correlation exponent (0.667
c'	$k_L a$ correlation exponent (-0.86)
F	nutrient feeding rate (0.0056 l/h)
H_c	Henry's law coefficient for CO_2 at 30° C (0.035 atm-l/mmol)
H_o	Henry's law coefficient for O_2 at 30° C (0.96 atm-l/mmol)
k_d	degradation constant for P (0.01 h^{-1})
kd_1	degradation for the Biomass (0.06 h^{-1})
$k_L a$	mass transfer coefficient (h^{-1})
K_I	inhibition constant for S on P (0.1 g/l)
K_{OP}	Contois constant for O_2 limitation of P (3.0×10^{-5} mmol/g)
K_{OX}	Contois constant for O_2 limitation of X (1.11×10^{-3} mmol/g)
K_P	Monod constant for S limitation of P (1.0×10^{-4} g/l)
K_X	Contois constant for S limitation of X (6.0×10^{-3} g/g)
m_o	maintenance coefficient for O_2 (0.467 mmol/g)
m_S	maintenance coefficient for S (0.029 g/g)
N_i	number of impellers (2)
p	operating pressure (1.0 atm)
P_g	gassed power (23.1 HP/1000l)
q_{in}	gas inlet flowrate (720.0 l/h)
R	Gas constant (0.082 atm-l/mol-K)
T	operating temperature (303 ° K)
v_s	superficial gas velocity (124 cm/s)
$Y_{P/C}$	Yield of P on C (0.2 g/g)
$Y_{P/O}$	Yield of P on O (0.2 g/g)
$Y_{P/S}$	Yield of P on S (1.2 g/g)
$Y_{X/C}$	Yield of X on C (0.037 g/g)
$Y_{X/O}$	Yield of X on O (0.04 g/g)
$Y_{X/S}$	Yield of X on S (0.47 g/g)
μ_P	Production rate for P (0.0055 h^{-1})
μ_X	Growth rate for X (0.11 h^{-1})

APPENDIX-B

Maple Source Code for Case Study 1 (Chapter 3)

```
# This is a maple input file used to analyse the observability properties of
# dynamic systems. Case Study 1 discussed in Chapter 3 of the thesis is
# considered here.

# The following line write the output produced by maple to a file called
# 'case1out'

writeto(case1out);

#
# The following line invokes the linear algebra capabilities of maple.
#

with(linalg);

#
# First formulate the dynamic balance equation for the biomass (Equation 3.11,
# 3.12 and 3.13). Note that the noise vector is ignored as observability is a
# deterministic property.

f1(b,myu,mc):=myu*b;
f2(b,myu,mc):=0;
f3(b,myu,mc):=0;

# Linearize the system around some latest estimate of the state vector X

F(b,myu,mc):=array([[f1(b,myu,mc)],[f2(b,myu,mc)],[f3(b,myu,mc)]]);
A1:=map(diff,F(b,myu,mc),b);
A2:=map(diff,F(b,myu,mc),myu);
A3:=map(diff,F(b,myu,mc),mc);
A:=augment(A1,A2,A3);
#
# Perform a simple Euler discretization
#
eye:=array(identity,1..3,1..3);
Ats:=scalarmul(A,ts);
Ad:=add(eye,Ats);
#
# Ad above is the discrete state transition matrix as defined in equation 3.15
# of the thesis
#
#  Now linearize the measurement matrix at the major sampling instant
#
```

```
h1(b,myu,mc):=b;
h2(b,myu,mc):=k1*myu*b+mc*b;
hmat(b,myu,mc):=array([[h1(b,myu,mc)],[h2(b,myu,mc)]]);
hc1:=map(diff,hmat(b,myu,mc),b);
hc2:=map(diff,hmat(b,myu,mc),myu);
hc3:=map(diff,hmat(b,myu,mc),mc);
#
# H below is the linearized measurement matrix defined in equation 3.16
# of the thesis.
#
H:=augment(hc1,hc2,hc3);
#
#
# Now construct the observability matrix as seen in equation 3.17
#
HA:=multiply(H,Ad);
HA2:=multiply(HA,A);
Ob:=stack(H,HA,HA2);
#
#
# Now symbolically check the rank of the observability matrix
#
#
rank_ob:=rank(Ob);
#
# The following line returns control of maple back to the command line

writeto(terminal);
```

Maple Output from the source code for case study 1 (Chapter 3)

```
Warning: new definition for   norm
Warning: new definition for   trace
[BlockDiagonal, GramSchmidt, JordanBlock, Wronskian, add, addcol, addrow, adj,

    adjoint, angle, augment, backsub, band, basis, bezout, blockmatrix,

    charmat, charpoly, col, coldim, colspace, colspan, companion, concat,

    cond, copyinto, crossprod, curl, definite, delcols, delrows, det, diag,

    diverge, dotprod, eigenvals, eigenvects, entermatrix, equal, exponential,

    extend, ffgausselim, fibonacci, frobenius, gausselim, gaussjord,

    genmatrix, grad, hadamard, hermite, hessian, hilbert, htranspose,

    ihermite, indexfunc, innerprod, intbasis, inverse, ismith, iszero,

    jacobian, jordan, kernel, laplacian, leastsqrs, linsolve, matrix, minor,

    minpoly, mulcol, mulrow, multiply, norm, normalize, nullspace, orthog,

    permanent, pivot, potential, randmatrix, randvector, rank, ratform, row,

    rowdim, rowspace, rowspan, rref, scalarmul, singularvals, smith, stack,

    submatrix, subvector, sumbasis, swapcol, swaprow, sylvester, toeplitz,

    trace, transpose, vandermonde, vecpotent, vectdim, vector]

                      f1(b, myu, mc) := myu b

                       f2(b, myu, mc) := 0

                       f3(b, myu, mc) := 0

                               [        myu b         ]
                               [                      ]
                    F(b, myu, mc) := [    0             ]
                               [                      ]
                               [        0             ]
```

```
                         [    myu    ]
                         [           ]
                A1 :=    [     0     ]
                         [           ]
                         [     0     ]

                         [     b     ]
                         [           ]
                A2 :=    [     0     ]
                         [           ]
                         [     0     ]

                         [     0     ]
                         [           ]
                A3 :=    [     0     ]
                         [           ]
                         [     0     ]

                         [    myu    b    0     ]
                         [                      ]
                A :=     [     0     0    0     ]
                         [                      ]
                         [     0     0    0     ]

eye := array(identity, 1 .. 3, 1 .. 3, [])

                     [    ts myu        ts b    0    ]
                     [                               ]
          Ats :=     [    0             0       0    ]
                     [                               ]
                     [    0             0       0    ]

                     [    1 + ts myu    ts b    0    ]
                     [                               ]
          Ad :=      [    0             1       0    ]
                     [                               ]
                     [    0             0       1    ]

          h1(b, myu, mc) := b

     h2(b, myu, mc) := k1 myu b + mc b

                         [           b                  ]
hmat(b, myu, mc) :=      [                              ]
                         [           k1 myu b + mc b    ]
```

```
                          [        1             ]
             hc1 :=       [                      ]
                          [        k1 myu + mc   ]

               hc2 :=     [        0             ]
                          [                      ]
                          [        k1 b          ]


                 hc3 :=   [        0        ]
                          [                 ]
                          [        b        ]

                 [       1        0      0       ]
         H :=    [                               ]
                 [ k1 myu+mc    k1 b     b       ]


          [        1 + ts myu                   ts b                            0       ]
 HA :=    [                                                                             ]
          [ (k1 myu + mc) (1 + ts myu)          ts b k1 myu + ts b mc + k1 b    b       ]

          [ (1 + ts myu) myu                    (1 + ts myu) b                  0       ]
HA2 :=    [                                                                             ]
          [ (k1 myu + mc) (1 + ts myu) myu      (k1 myu + mc) (1 + ts myu) b    0       ]

          [ 1                                   0                               0       ]
          [                                                                             ]
          [ k1 myu + mc                         k1 b                            b       ]
          [                                                                             ]
          [ 1 + ts myu                          ts b                            0       ]
Ob :=     [                                                                             ]
          [ (k1 myu + mc) (1 + ts myu)          ts b k1 myu + ts b mc + k1 b    b       ]
          [                                                                             ]
          [ (1 + ts myu) myu                    (1 + ts myu) b                  0       ]
          [                                                                             ]
          [ (k1 myu + mc) (1 + ts myu) myu      (k1 myu + mc) (1 + ts myu) b    0       ]


                    rank_ob := 3
```